

nature

15 September 1977

Destination without route-map

NOSTALGIA, as more than one wag has said, is not what it used to be. Nor, according to Denis Hayes, is the future. The entire world, he argues in his Worldwatch Institute book *Rays of Hope* (published this week in the United States*), stands at the edge of an "awesome discontinuity" in its production and use of energy—hence the book's subtitle, 'The transition to a post-petroleum world'. In trying to anticipate what that world could look like, it is undoubtedly an important sketch of a growingly complex picture; its flaw, if that is what it is, is that it ignores how the disputed views of the final canvas are to be reconciled and implemented.

Firmly in the now well-established tradition of Worldwatch publications, the lucidly written and remarkably broad-ranging exposition is a valuable synthesis of existing work, here in the burgeoning field of energy—indeed, it is a synthesis of a synthesis, incorporating Hayes' already-published arguments on conservation, nuclear power and solar energy. Designed also to influence as well as inform, it has a clear message to convey: the three energy sources on which to build a self-sustaining post-petroleum world, namely coal, nuclear and solar, have narrowed to just one—the sun; the technology is already available, now is the time to move.

Part of the argument is familiar enough. The fossil fuels outlook is discouraging: without a change, the current generation will consume most of the oil and gas. Resource, environmental and climatic constraints make coal just a transitional fuel. The nuclear outlook is even less encouraging: the threats of proliferation and terrorism come on top of problems of radiation, waste, uranium resources, breeders and safety. With fusion's promise weak, that leaves the safe sustainable sources—the sun, wind, water and 'plant power'—and a crying need for conservation.

To see it simply as a 'pro-solar' or as an 'anti-nuclear' book would be a mistake, however, though it is this almost to a fault and as convincing as anything that has taken the opposite point of view. The book's relentless onslaught on the wasteful use of energy, specifically in food production, transport and construction, together with its detailed technical picture of a more energy-efficient world is especially welcome. So too is its historical and global perspective, although parts

are plainly pitched to a US audience.

Where the argument is different, but also troubled, is in the character of its advocacy. Worldwatch's president, Lester Brown, says in a foreword that the question is not whether or not we make the transition, but whether it will be smooth or chaotic. Unfortunately Hayes seems to believe it is better to arrive than to travel, for he is as short and unenlightening on how politically to secure his alternative as he is long and rewarding on what, in energy terms, the world ought to look like under it.

On a subject this controversial, this is a rather glaring omission. To consider global energy problems without considering the global political economy is like scaling a mountain without allowing for the weather. But few books go even as far as Hayes does. It also remains unfortunately true that people let the unpredictability of the weather mask their appreciation of mountains, and he has undoubtedly taken account of this. Yet in a book whose underlying theme is world-wide change, questions are bound to go begging when the technical changes are teased out while the concomitant social changes are not. And that is exactly what happens.

We are told—but only told—of the 'weighty' depletion issue, which focuses on the discount rates that the markets and the politicians apply to, say, a future barrel of oil. We are told of the highly centralised technocratic and authoritarian societies that nuclear power fosters. We are also told that there is neither a 'quick fix' nor a *deus ex machina*, that the coming transition is unlikely to be smooth and painless, that there will be opposition. And we are told that the issues are not scientific and technical, but social, political and philosophical—that redistributions of land and of wealth must be enormous, both within and between countries.

This too will demand some authoritarian action, for, historically speaking, a more explosive political issue is difficult to find. But for Hayes it must be the subject of another book. So we are left with rays of hope instead of straws, and an argument for a least bad option. It is a pity. Perhaps, as he intimates, civilised societies must discover their own solutions and carve out their own paths. But in an interdependent and competitive world, events look more and more like intervening to do their own damage. This book was needed five years ago. Even with it, people will regret not being institutionally prepared as well as technically competent to survive the impending threat of holocaust. □

*Denis Hayes, *Rays of Hope: The Transition to a Post-Petroleum World* (W. W. Norton, September 1977) \$3.95



A good time to raise standards

EVERY year we scan the statistics of The Universities Central Council on Admission (UCCA) for trends, and occasionally we get rapped over the knuckles for misinterpretation. This year's figures, just out, offer the usual happy hunting ground.

The statistics, as always a year late, refer to those who applied during the year 1975-76, generally for entry in October 1976. They show applications up again to around 142,000. Overseas candidates, particularly from the Commonwealth, continue to grow in number more rapidly than home candidates—in 1976 they accounted for one in seven of all applicants. Places were found for 74,000 applicants; the figure for total applicants, which is always about twice the number accepted, includes a large number who subsequently fail to gain adequate school qualifications, who withdraw, who are applying for the second time and so on. So it must not be inferred that there is a genuine shortage of places.

Engineering continues its run of popularity (which started well before the recent governmental campaign). Projections to 1977 suggest there are now at least 40% more applying for engineering than in 1972. And even mathematics, physics and chemistry—which lost popularity until 1975—may now be sharing again in the general rise in applications.

Simple head-counting, however, conceals some striking variations in quality. The relative paucity of candidates in mathematics, physics and chemistry does not seem to have led to a diminished standard in those accepted—at least as far as paper qualifications go. Indeed the school performances of accepted physicists rival those of accepted medical students—the latter a category in which universities can be very selective. Good scientists still seem to be coming forward. The position is somewhat different in engineering. Rising quality has not gone along with rising numbers, which may mean that schools have been urging more of their modestly accomplished pupils to consider engineering.

It is against this background that one must consider the suggestion made in the report that universities may soon have to allocate further resources to alleviate the 'critical situation' which seems to be developing in engineering places. Only two or three years ago, of course, we were being told by the engineers of half-empty departments: it is hard to believe the turn-around has been that dramatic. But maybe the universities, rather than create more spaces, should become more selective as time goes on, in the hope of creating a genuine impression amongst students not just that the country welcomes engineers, but that standards in engineering are high. □

Research funding: the new trichotomy

William S. Hillman comments on the relevance of the dichotomy of 'pure' and 'applied' science

THE dichotomy contrasting 'basic' and 'applied' research is still commonplace in discussions on funding. Such discussions often conclude that one mode is over-supported at the expense of the other. Yet on most reasonable definitions of the two, history suggests that neither prospers alone. The old dichotomy is at best misleading, and distracts attention from a more immediately dangerous aspect of current policies. That aspect invites analysis by trichotomy, if you will excuse such language.

Strictly speaking, nothing in the current situation is entirely new. Reasonably enough, the patrons of science have always wanted useful results, quickly if possible. But the main dispenser of patronage now is government, with its self-justifying administrative hierarchies, and it has finally become clear that the taxpayers' resources are finite. In addition, occasional spectacular successes, such as moon landings, have been inappropriately but invidiously contrasted with (for example) the 'failure' of NIH research to abolish cancer. Recent versions of the king's desire for the alchemists' gold have thus split research into at least three recognisable types: real, imitation (or administrative) and defensive. The terms are not quite self-explanatory, but they correspond roughly to real gold, fool's gold and the elusive quicksilver.

Real research is originated, organised and carried out by investigators who understand the field in question. It is aimed at problems which in the view of those investigators are both important and reasonably likely to reward the effort with some return in understanding, control or both. The 'applied/basic' dichotomy is meaningless here. A salient characteristic of real research is that it engages the best judgment of capable scientists. The subjective elements in this description (who is 'capable'?) do not obscure the distinction between it and the second category.

Imitation research, in contrast, starts with the belief that, because science has proved useful in the past, scientists should be able to do anything, right now, given the funds, proper organisation and (most important) what sports writers call desire. This is all regardless of whether the necessary concepts, techniques and people actually exist. The primary motive for organising imitation research is neither controlling nor understanding nature, but pleasing one's superiors. That those superiors should know little about the opportunities and limitations in specialised fields is inevitable, but many organisers of imitation research either share that ignorance or are simply cynical. Hence manifold reports, detailed timetables and complex flow charts are prominent features of the game, leading naturally to the synonym 'administrative research'. The overriding rule is that the players must avoid being corrupted by luxuries such as intellectual rigour, imagination and patience; to that end, frequent exercises in relevance, interdisciplinary communications modalities and urgency are scheduled.

What, finally, is defensive research? The notion is analogous to that of defensive driving in that both recognise the hazards of assuming people will behave sensibly; or to the developing style of defensive medicine, in which procedures that are legal safeguards for the physician may be wasteful for both the patient and society. Defensive research, in short, is what so many scientists must do who would prefer to be doing real research but lack private incomes. The result is that a core of real research becomes surrounded with a set of possibly quite worthless elements designed to impress those who want the gaudy imitation rather than await the real thing.

There is no space here for examples, but few readers will need them. Very likely some policy-makers, here and there, in one agency or another, will indignantly deny any knowledge of imitation or defensive research. But they are easily answered with a paraphrase of the ancient epitaph: if you need examples, look around you. □

From détente to development

Eric Burhop reports on the discussion between scientists at the 27th Pugwash Conference

ATTENDANCE at the 27th Pugwash Conference, held in Munich on 24–29 August, was as always on an individual basis, facilitating the frank and uninhibited exchanges between scientists of very different backgrounds that has always been a feature of Pugwash. Two hundred and sixty participants from 54 countries met to discuss problems of universal concern: détente, human rights, energy and development. About 50 of them came from countries of the Third World. Those not attending on an individual basis included 18 observers representing governmental international organisations.

In his address to the opening session, Herr Matthöfer, Minister of Research and Technology of the Federal German Republic, remarked "Today Pugwash is a very familiar name, indeed a symbol of the responsible active efforts of scientists from all over the world to help solve the major world-wide tasks of humanity across political and ideological barriers".

For those who may not be so familiar with the history, Pugwash is the name of a small Nova Scotian village, birthplace of the Cleveland, Ohio, industrial magnate, Cyrus Eaton, who financed the first of these conferences held there in July 1957. The call for the holding of such a conference came in a remarkable statement drafted by Bertrand Russell in his incomparably clear and compelling language and beginning: "In the tragic situation which confronts humanity, we feel that scientists should assemble in conference to discuss the perils that have arisen as

a result of the development of weapons of mass destruction . . .". *The statement goes on to spell out to the world the grave and immediate dangers to the whole of humanity implicit in the development of the hydrogen bomb. It was signed by Einstein only two days before his death in May 1955 and by nine other eminent scientists from six countries. The Russell–Einstein Manifesto, as it has come to be known, was presented to the world at a press conference presided over by Joseph Rotblat and held in Caxton Hall, London, in July 1955.

Two roles

There have always been two conceptions of the aims of the Pugwash Conferences. The Russell–Einstein Manifesto looked to them to rouse public opinion to the dangers of the development of nuclear weapons by issuing statements which, coming from scientists of such eminence and integrity, could be ignored neither by peoples nor by governments. The other concept of the role of Pugwash as providing facilities for private discussions between the very experts from NATO and Warsaw Pact countries who were advising their governments on problems of weapons development and disarmament, was especially associated with the thinking of Leo Szilard.

Both Pugwash roles have had an important effect on relations between the two power blocs in the past. The mutual understanding of each other's thinking has made remote the possibility of stumbling into nuclear war by accident, through, for example, wrong interpretation of observations on a radar screen. Pugwash Conferences have been credited with playing a significant role in achieving the Partial Test Ban and Non-Proliferation Treaties and in the development of the theory of deterrence with its strange array of concepts such as first and second strike capability, mutually assured destruction and all the rest.

The general conferences are held

every year, the participants from each country or region being selected by local Pugwash groups. Every fifth year the whole Pugwash community, made up of every participant in previous Pugwash meetings, is eligible to attend. This quinquennial conference elects the principal officers and council of approximately 25 persons that will direct the work for the following five years, assesses the work of the previous quinquennium and plans the future programme. The Munich Conference was a quinquennial one, indeed the largest yet held.

Customarily the conferences work mainly through working groups which deal with specific topics, preparing reports for plenary sessions in the concluding stages of the conference. At the Munich Conference there were eight such groups dealing with nuclear arms control and disarmament; arms control and disarmament in the non-nuclear realm; coexistence, détente and cooperation between nations and systems; security of developing nations; development problems of the economically poor nations; energy, world resources and population trends; environmental hazards of global concern; science, scientists and society.

The prevailing judgment of the first working group was that the SALT talks are not progressing fast enough to prevent advances in military technology which negate the effect of agreements, based on SALT, already reached. The time for stopping the arms race is running out. There were powerful pleas to examine again basic concepts such as that of deterrence which can at best provide a breathing space. Pugwash should take the lead in pressing for a start on real disarmament and not restrict its approach to arms control measures.

Almost unanimous concern was expressed by several groups about President Carter's decision to go ahead with the development of the neutron bomb. The idea of a weapon specifically designed to kill people while preserving property largely intact has caused a wave of revulsion and it was suggested that Pugwash could recapture some of its old sense of urgency in an appeal to public opinion on issues such as this and on the reported development of a nuclear capability by South Africa.

Public statement

It is the practice of Pugwash that its conferences, symposia, workshops, etc, issue reports of discussions and recommendations for the benefit of Pugwash members but public statements formulating specific policies on such matters can come only from Council. (See box for the statement issued by Council on these questions,

Eric Burhop is Professor of Physics at University College, London, and President of the World Federation of Scientific Workers.



Russell at the 1955 press conference

**It is not widely known that originally Russell was himself sceptical about calling a conference. He envisaged simply the issuing of a statement. The proposal to add the call to scientists to meet in conference was made by Frédéric Joliot-Curie on behalf of the World Federation of Scientific Workers, of which he was President, in a letter to Russell in January 1955. The writer acted as go-between in the negotiations between Russell and Joliot-Curie.*

reflecting the discussions at the Munich Pugwash Conference.)

The discussions on détente predictably led on to problems of human rights in an atmosphere largely free of acrimonious interchange of charge and countercharge. Appalling departures from acceptable standards are reported from many countries while no country seems faultless. However, the approach to human rights problems should be guided by how to help the victims. They have been used all too often for propaganda purposes or even to attempt to destabilise governments. Discussions on human rights issues tend to be simplistic, the fact that human rights cannot be separated from human responsibilities being forgotten. Nor can the question be adequately discussed in isolation from its social and political framework.

The question of energy supplies practically dominated the discussions on world resources. There was general agreement that ultimately energy needs must be met from solar, wind, geothermal and safe nuclear fusion power but no consensus about how we are to get through the next 40 or 50 years. There was some but not much enthusiasm for the fast breeder solution and a feeling that there was still some time available before committing us to a full programme of fast breeder reactor development. If a determined programme of energy saving were coupled with control of population growth, one could realistically aim for

a situation in which, by the year 2020, the world population would not rise above 6.7×10^9 with an average energy use of 5 kWt (kilowatts-thermal)—8.0 kWt (rich nations), 3.2 kWt (poor nations)—within a global rate of energy use of only four times that of today. It is believed such a level of demand could be met without introducing breeder technology.

North-South divisions

The problems of development loomed large in the conference discussions. Indeed, any differences in the conference were far more between North and South than between socialist and non-socialist countries. Many delegates from the Third World do indeed feel that their problems are inadequately dealt with by Pugwash and stress that problems arising from the policies of the industrialised countries relative to the Third World, the role of multinational companies, and also to a lesser extent contradictions between Third World countries themselves may represent the most serious threat to peace. On the other hand, some participants looked back somewhat nostalgically to the early Pugwash Conferences when participants were all more or less expert in some aspect of the field of nuclear weapons and it was possible to issue sharp, incisive statements in this field, carrying great weight. A few felt that there would be a case for two separate types of Pugwash Conferences, one specifically de-

voted to disarmament and the other to Third World problems. This would surely be a retrograde step and could only sharpen North-South divisions and the overwhelming view was in favour of continuing as at present.

Pugwash has developed a certain mystique and influence of its own. Some may think it elitist and self-perpetuating; some may chafe at the confidentiality of the discussions, which is an essential feature of the whole Pugwash concept, but it seems that Pugwash is a powerful force for peace and understanding between nations. Thanks to a band of devoted scientists, too numerous to mention but from whom at the present time could be singled out Joseph Rotblat, Bernard Feld, Martin Kaplan, Moishe Markov and Dorothy Hodgkin, the ideas of Russell and Einstein have remained alive and the Pugwash movement has become a living movement with its members showing a genuine sense of loyalty to these ideas and of belonging to the movement.

Of the tasks ahead special emphasis should be placed on the participation of Pugwash in two vital UN meetings: the Special Session of the UN General Assembly on Disarmament in the spring of 1978, and the UN Conference on Science and Technology for Development in the summer of 1979. In both these areas the world community has grievously failed in the past. Here is perhaps an opportunity for a new beginning. □

Declaration by the Pugwash Council

In the light of discussions at the 27th Pugwash Conference on Science and World Affairs, the Council of the Pugwash Movement feels impelled to issue the following declaration:

The world is poised for a new, more intensive and more dangerous round of the arms race. Three features of the world scene account for our sense of heightened urgency and danger:

New weapons of mass destruction. The neutron bomb is proposed for development in the heart of Europe. It is sometimes called a 'clean' or damage-free bomb. On the contrary, both its lethal radiation effects and the short and long-term biological damage it would cause are substantially greater than for existing nuclear weapons of comparable size. There would be strong induced radioactivity from neutron capture in the soil. Because of its relatively small size, it would narrow the distinction between conventional and nuclear weapons and thereby make the use of nuclear weapons more likely.

But the neutron bomb is only one example of new types of weapons now coming into military arsenals: cruise missiles, mobile ballistic missiles, and

others. These are often provocative and thus destabilising. Their deployment is not verifiable with national means, and thus frustrate efforts at control. And the number of nuclear warheads continues to mount. *The deployment of all these new weapons must be stopped.*

Proliferation. There was serious concern at the conference over reports of an imminent nuclear-weapons test in South Africa. This concern was not allayed by the assurances by the South African Government that it has no intention to do so. The acquisition of nuclear weapons by South Africa would be a grave peril to the peoples of Southern Africa and the rest of the world. Developments there must be kept under intense continuing surveillance. Any collaboration with South Africa in the nuclear weapon field—whether on the governmental, commercial or scientific level—must stop. The United Nations should be urged to apply effective sanctions should South Africa be proven to be developing nuclear weapons.

But South Africa represents only the most urgent and immediate occasion for concern. At various points around the globe there have been, from time to time, disturbing reports of states seeking

nuclear weapons. We unequivocally condemn moves towards further nuclear weapon proliferation.

The failure of arms control. In spite of endless efforts, progress towards limiting armaments and stopping the arms race has been all but invisible. Everywhere there is a new sense of impasse. And this is particularly ominous in the SALT and MBFR negotiations. There is no military reason for their failure to make progress. No nation's security would be endangered, indeed it would be enhanced, by much lower levels of armaments. The obstacles are political and it requires only political will and political decisions to overcome them.

● We call on the heads of concerned governments and states all over the world, particularly of the USA and USSR, to halt new weapons deployment and reverse the arms race.

● We call on men and women everywhere to redouble their efforts to make their governments understand and act in the face of our common peril.

And we in Pugwash rededicate ourselves to the achievement of a world at peace.

GEOS

Salvaged results

Stuart Sharrock reports on some of the preliminary results from experiments aboard Geos

At a champagne breakfast on 20 April at Cape Canaveral, European Space Agency officials were gloomy. Instead of celebrating the successful launch of the Geos satellite they were contemplating the total failure of the whole mission. But within a few days the Geos ground control at Darmstadt had partially rescued the situation—although not in the planned geostationary orbit, the satellite would at least be spending some time in the right place.

This would have been a hollow victory, however, if it had meant no scientific results, so the experimenters had to retrieve what they could. In Munich last week they met to discuss their preliminary findings. Although there are some problems, remarkably, there are already some interesting results. After only four months it is clear that much of the scientific programme is going to be a success. But the role of Geos as the reference spacecraft of the International Magnetospheric Study (IMS) cannot be restored and the success of the experiments makes it imperative that the second spacecraft should be launched into geostationary orbit in the near future.

Geos was planned as the first geostationary satellite devoted solely to magnetospheric research. It followed

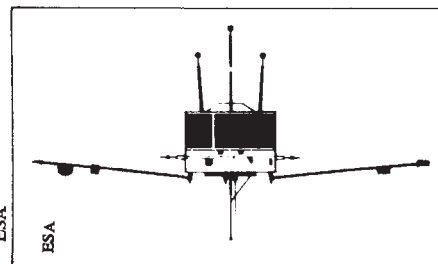
the ATS series of satellites which confirmed that a geostationary orbit was ideal for studying the magnetosphere. The intention was to launch Geos 1 into geostationary orbit early in 1977; the second identical satellite, Geos 2, was to be launched into an elliptical geosynchronous orbit in December 1979 by Ariane, ESA's own launch vehicle now under development. The second mission, codenamed Geosari, was to be complementary to the first, providing a wider spatial coverage of the magnetosphere. The only changes to the second payload would be improvements to the experiments found necessary as a result of experience gained during the first Geos mission.

The attractiveness of Geos as a tool for investigating the magnetosphere is based on the uniqueness of its planned orbit and the sophistication of its payload. Its position was planned to lie on magnetic field lines connecting northern and southern auroral zones. In addition, its geostationary orbit would have allowed average conditions to be established, deviations from the average indicating temporal rather than spatial variations. As the near-earth environment is not static this discrimination is important, but it can only be fully achieved by simultaneous use of more than one satellite such as the pair of satellites of the ISEE mission due for launch in October.

Although Geos has lost these advantages because it is not in geostationary orbit the possibilities opened up by the comprehensive nature of the experiment package still largely apply. It is still possible during part of Geos' new orbit to make simultaneous measurements on waves and particles whilst monitoring the background electric and magnetic fields and plasma density.

When the malfunctioning Delta launch vehicle failed to place Geos into the correct transfer orbit it was clear that injection into a geostationary orbit was not possible. ESA's Space Operations Centre at Darmstadt however managed to place it into a 12 h elliptic orbit with an apogee of around 7 earth radii using the apogee boost motor (see diagram). The orbit was chosen because the satellite is visible around apogee for about 10 hours per day from the ground receiving station near Darmstadt.

Initially the local time of the apogee of the orbit was in the evening sector, but it gets progressively earlier, drifting at a rate of 1 hour every 17.5 days. It has just passed the earth-sun line and



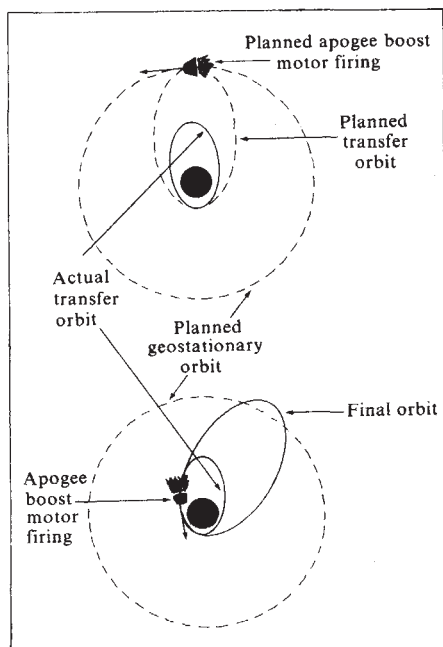
will start to move into the night sector around Christmas. The aurora are much more active in the night sector and so any correlated measurements with rocket campaigns will have to wait until 1978.

So far each experiment has been operated independently and there are still a few interference problems to be sorted out before the optimum mode of automatic operation of the experimental package will be known. But the initial data indicate that the Geos payload is sophisticated enough to provide reliable measurements of electric fields and plasma densities outside the plasmasphere. All data obtained so far are from the afternoon sector of the magnetosphere.

In perhaps the most ambitious experiment on board, an electron beam is fired from the satellite perpendicular to the local magnetic field (determined by another Geos experiment). It is deflected by the geomagnetic field in a circular path of several kilometres radius to return to the satellite close to its source. The distance between the source and the point of return depends on the electric field and the gradient of the magnetic field; the two contributions can be sorted out by varying the energy of the emitted electrons and the plasma drift velocity and direction can be determined. This is difficult to verify on earth as a vacuum chamber a few kilometres long would be required in a magnetic field of less than 100 γ . Unfortunately after about one week of operation the oxide cathode of the electron gun became poisoned but, even with only a few days data, the viability of the method was proved.

Another experiment measuring electric fields—in line with the Geos philosophy of providing redundant measurements of important parameters—has also been successful and interesting slow fluctuations have been seen which have yet to be correlated with data from the ULF wave experiment. The electrostatic wave experiment has been able to detect electrostatic waves with the help of information on the plasma frequency provided by an exploratory experiment using the first relaxation sounder to be launched above the ionosphere.

A variety of electromagnetic wave



Geos orbits: planned and achieved

phenomena have been observed, and some have been related with measurements at ground stations. It will be interesting to correlate these results with those from the various particle experiments but there are still some problems. Confusion over the number density and temperature of the plasma, however, may well be resolved by a determination of the energy spectrum of the various particles, but it needs further investigation of the contribution of the observed photoelectron cloud from the satellite itself and of the effects of spacecraft changing. These problems may be solved later in the year when Geos goes into eclipse.

Some experimenters have been unable so far to completely understand their results through the lack of reliable attitude data necessary to sort out spin modulation effects. But it should be only a matter of time before the relevant analysis programs are fully working.

The experiments measuring high energy particles are least affected by these problems. Preliminary results from a sophisticated mass spectrometer for measuring ion composition, indicate the presence of the doubly charged ions $^4\text{He}^{++}$ and $^{16}\text{O}^{++}$ deep in

the magnetosphere. The new orbit of Geos is almost a bonus in this case because ion compositions can be measured as a function of distance from the earth. Another unexpected bonus of the new orbit is the fact that Geos now spends considerable time near the geostationary satellite ATS-6. Earlier attempts to persuade NASA to drift ATS-6 past the planned geostationary position of Geos were unsuccessful but now the possibility of correlated measurements with similar instruments on the two satellites has become a reality.

The progress that has been made in such a short time after the disappointment of the failed launch is impressive and prospects for the future look bright. But most of the Geos programme in which coordinated measurements were planned with ground-based campaigns has had to be abandoned. Indeed the rocket campaigns from northern Sweden have been persistently plagued with bad luck. The rockets are supposed to pass through the transient auroral arcs and correlate their measurements with satellites poised at magnetically conjugate points above the equator. On the first launch ATS-6 was in position but the rocket exploded.

Geos should have been in position for the second rocket but delays had pushed back the Geos launch date and it was still on the ground. ATS-6 had meanwhile moved on. These difficulties are increased by the refusal of Norway to allow the rockets to overfly their territory and the consequently restricted rocket range makes it very difficult to hit the aurora. There are two rockets planned for 1978 and the rocket team hope their luck will change and that the second Geos spacecraft will have been launched into geostationary orbit.

Geos 2 has already been refurbished and will be of flight standard by the end of the year. It can either be launched into geostationary orbit by NASA early in 1978 using another Delta launcher, or it could wait until late 1979 to be sent up on ESA's own launcher Ariane. An early reflight on a Delta is what the experimenters want—from the way they have evaluated their data in such a short time they have in a sense earned it—and from some view points this appears the most likely option, but the extra cost to ESA will be about \$14 million and it is unclear where this will come from. ESA is expected to decide early in October. □

USA

Opening a biological pathway

The signing of the Panama Canal Treaty last week has implications for the ecology of the Atlantic and Pacific Oceans. Colin Norman reports

AMID the diplomatic fanfare and loud political protest which greeted the signing last week of the Panama Canal Treaty, muted rumblings of a scientific controversy were also faintly audible. The controversy centres on a little-noticed article in the Treaty committing the United States and Panama to conduct a joint study of the feasibility of building a sea-level canal within a few miles of the existing waterway.

The idea of carving out a direct, sea-level link between the Pacific and Atlantic Oceans has been investigated many times in the past, but political, economic and technical problems have so far kept it from becoming a reality. In fact, in spite of the renewed official interest in the idea, it is still considered unlikely that the channel will ever be dug. Nevertheless, the possibility is worrying some marine scientists, who fear that a sea-level canal would damage the ecology of the surrounding oceans.

The worry stems from the fact that

the tropical Pacific and Atlantic Oceans have been separated for perhaps five million years, since North and South America were joined by the isthmus or Panama. Consequently, marine life has evolved independently in the two oceans for long enough to ensure that few species are common to both. Blasting a sea-level link across Panama would open a biological pathway which may result in the migration of plants and animals into a new environment, a process which some marine scientists believe could have considerable impact on the oceans' ecology.

Very little migration takes place through the present canal because a freshwater lake in the middle of the waterway provides a barrier to most marine life, and the large number of locks along the canal means that there is little flow of water through the system. A sea-level canal would, however, be an entirely different prospect. Tidal ebb and flow through the canal, coupled with the fact that the average sea level on the Pacific side is slightly higher than that on the Atlantic side, could result in the transport of plants, animals, larvae and other organisms through the channel.

Among the more dramatic consequences that have been predicted as

a result of breaching the land barrier are the possibility that the yellow-bellied sea snake, a poisonous reptile which now lives only in the Pacific water, could become established in the Caribbean, and that the crown-of-thorns starfish might similarly invade the tropical Atlantic coral reefs. Other potential worries are that fish travelling through the canal might carry pathogens or viruses which are not known to native species. The central problem in such interchange is that the introduction of flora or fauna into a new environment might upset the ecological balance because there may be no natural predators and native species may not have evolved an effective defence mechanism against the newcomers.

Such possibilities are, of course, highly speculative, and some marine biologists contend that it is very unlikely that major environmental damage would result from opening a sea level channel. Nevertheless, there are several sobering examples of previous experience with the introduction of animals and plants into a new environment. For example, the building of a canal to bypass the Niagara Falls, providing a channel joining the Great Lakes to the Atlantic, has resulted in invasion of the lakes by a predatory fish-like creature called the sea lamprey. The lamprey has spread throughout the Great Lakes,

and decimated native species. Similarly, the introduction of the European rabbit into Australia and New Zealand was followed by a population explosion which caused serious damage to vegetation and topsoil.

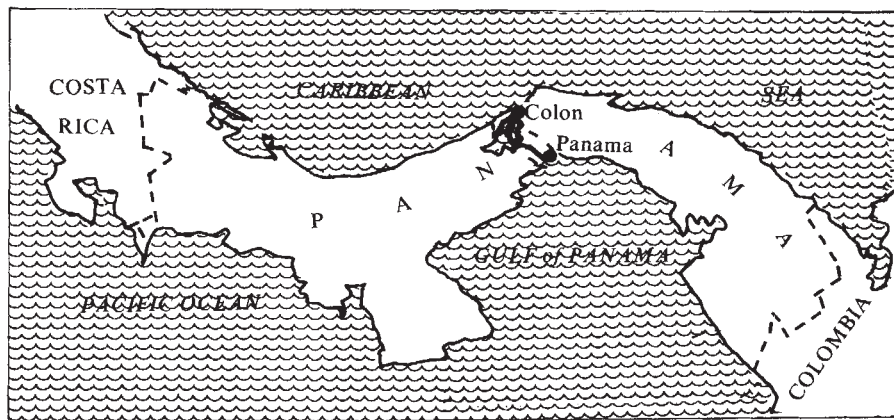
Such concerns were given wide publicity in the late 1960s, when the sea-level canal last came under serious study. A Presidential Commission, established in 1964, conducted a major feasibility study of such a canal, examining potential routes, engineering problems and the possibility of using nuclear explosives to blast out a channel. In 1970, the Commission recommended that the project should go ahead, along a route about 10 miles west of the present canal. Excavation should be by conventional means, the Commission said, and it calculated that the effort would cost about \$2,900 million in 1970 dollars.

Because of the high price tag, and in view of political problems in re-negotiating the existing Panama Canal Treaty, the Commission's recommendations were never implemented, and the whole idea swiftly dropped out of the limelight. It is, in fact, something of a surprise that the idea has been resurrected in the new canal treaty.

As part of the Commission's study, the National Academy of Sciences and the Battelle Memorial Institute were asked to conduct independent analyses of the likely ecological impact of opening a sea-level link between the Pacific and the Atlantic. The Academy's report, which was published as an appendix to the Official Commission Report, must rank as one of the most forthright statements of concern ever to emerge from that august institution. Calling the sea level canal "a gigantic natural experiment" which will have "unforseeable" consequences, the Academy called for a massive study of the tropical Pacific and Atlantic ecosystems before the project is allowed to go ahead. "Available information is altogether insufficient to allow reliable predictions of particular events resulting from the excavation of a sea-level canal in Panama", the Academy said.

The Battelle report was, however, more sanguine about the impact, noting that although there is scant knowledge of the ecology of the region, there is "no firm evidence to support the prediction of massive migrations from one ocean to another, followed by the extinction of thousands of species". The Commission itself was even less impressed with the warnings of disaster. It devoted a mere four pages of its report to a discussion of environmental concerns and concluded that the hazards, whatever they may be, would be "acceptable".

The argument for building a sea-level



Sorry, for copyright reasons some images on this page may not be available online

Camera Press

Miraflores locks, preventing flow between Atlantic and Pacific

waterway is simply that the present canal is already heavily travelled and it is likely to reach its capacity in the 1990s. Moreover, its locks are too small to take the large freighters and supertankers which are now appearing on the oceans. Such limits were, in fact, realised, back in the 1930s, when work was begun on the construction of a series of larger locks alongside the present canal, but construction was stopped during the war and it was never resumed. (The new Panama Canal Treaty in fact calls for a study of the feasibility of completing the construction of the old locks as an alternative to building a new sea-level canal.)

More recently, a new argument has appeared: a sea-level canal will be needed to transport Alaskan oil and gas to the east coast of the United States. According to this argument, which has been advanced most prominently by Senator Mike Gravel of Alaska, the cost of building a new canal (about \$5,900 million at today's prices, Gravel reckons), should be measured against the cost of building a new pipeline system to transport Alaskan oil and gas from the oil-rich west coast to markets in the east. Once all the advantages are counted up, Gravel reckons that the canal will seem like a bargain. He has stated that he will seek congressional approval for the 1970 report to be up-

dated.

In anticipation of such renewed interest, Dr Frank Press, President Carter's Science Adviser last month asked the National Academy of Sciences to take a fresh look at its earlier report to the Commission to see whether recent scientific data are available to help predict more precisely the likely environmental consequences. Last week, the Academy Committee held two days of hearings and it is hoping to produce a report for Press by early October. Judging from last week's meeting, the Committee is likely to conclude that although a good deal of work has been done, knowledge of the Panamanian ecology is still too sparse to predict with certainty the impact of breaching the land barrier between the tropical Pacific and Atlantic oceans.

Asked last week when the joint US-Panama study envisaged in the treaty is expected to get under way, a State Department spokesman said that no details have yet been worked out. The first priority will be to steer the treaty itself through conservative shoals in the Senate. In view of the immense political problems involved in re-negotiating the treaty, the prospects for negotiating a new treaty to build a sea-level canal in Panama must, at this point, be considered remote. □

USSR

● Training enough highly qualified scientific manpower is a major problem of the Soviet economy. According to the current five-year plan, some 9.6 million new engineers, technologists, agronomists, doctors and other specialists will enter the labour pool by 1980, with increasing emphasis on graduates in molecular biology, environmental protection, atomic and electronic technology, electrical instrumentation, automation, agriculture and forestry. To train them the Soviet Union now has 859 universities and other higher educational institutions, and 4,303 technical colleges. The problem is not simply one of training, however; the proper deployment of scientific personnel is a constant topic of discussion.

Current planning policy calls for closer integration of research in industry. One way to bring this about is to involve university students in projects with an immediate industrial application. Speaking at the opening of the new Grodno State University last month, S. M. Carnau, Minister of Education of the Byelorussian SSR, noted with approval the "widespread attraction of students of senior courses" towards choosing as their term or diploma project a specific contract for research from an industrial enterprise or organisation. This, he said, has considerably extended the "geography" of application of young scientists' talents.

One potential source of close association between academic life and industrial needs would seem to be through the considerable numbers of external and evening students. In the present academic year, the 'full-time' university and technical college intake is estimated by *Pravda* as "more than a million". If the demand for new scientific personnel during 1981-85 equals or exceeds the 9.6 million of the current five-year plan (as all available figures indicate), and assuming a current annual university intake of 1.2 million, by the early 1980s almost one-third of the new graduates will come from external and evening courses.

Unfortunately, these 'part-time' graduates do not necessarily return to industry. The current 'press discussion' of the new Draft Constitution recently included a long letter from a certain Senior Engineer A. Akimova from Khabarovsk, suggesting that 'part-time' graduates should be compelled, by law, to return to their former 'collective'. Ms Akimova complains that in spite of the self-sacrifice of their fellow-workers, who

have to cover their duties when they are absent on paid leave taking examinations, and who "try to provide the conditions of study" during work breaks, many young graduates "on receiving the diploma of a specialist, straight away try to find a



more advantageous job". Ms Akimova's letter, like the majority of contributions to the 'debate', takes the form of a shop-floor proposal, but also like the majority of such letters, has wider implications than the complaint of a few disgruntled workers. It would clearly be to the advantage of the managers of industrial enterprises if they could be sure that the promising young workers involved in part-time education would remain at the disposal of their own production team after graduation, instead of disappearing into the general scientific labour pool.

● This year marks the fortieth anniversary of the first 'North Pole' drifting ice-floe station, in which a five-man team drifted for 274 days in the vicinity of the Pole. That expedition established a valuable research procedure which still continues; this summer 'North Pole' 22 and 23 continued the tradition.

In comparison with these long-term voyages, the recent dash to the Pole by the 'Arktika' icebreaker appears to have had only a limited role in gathering meteorological, geophysical and oceanological data. The expedition was clearly considerably prestigious, and its heroes will doubtless figure prominently in the forthcoming 60th Anniversary Celebrations of the October Revolution. But all in all, only 15 h were spent at the Pole, which included time spent in ceremonial flag-hoisting, rocket-firing and holding hands round the pole.

The main scientific value of the voyage would appear to relate to the design and performance of the

'Arktika' herself. According to A. I. Fokin, Deputy Minister of the Shipbuilding Industry, 100 scientific research institutions were involved in the design of the ship and equipment. A new navigational procedure was tested—the use of helicopters instead of the traditional crows'-nest lookout, to locate the best route through the ice. It is claimed that as a result, speed increased 50-100% and the 'Arktika' reached the Pole seven days ahead of schedule, even though the floes were at times 4 m thick. A new and even more modern icebreaker, the 'Sibir', whose cabins will have independent suspension, obviating the effect of hull motion, will be completed shortly.

The northern sea route, between European Russia and the Soviet Far East is of considerable importance to Soviet economic planning. Commenting on the success of the 'Arktika', Timofei Guzhenko, the leader of the expedition and Minister of Merchant Marine remarked that the commercial use of the route is now a "real possibility". The immediate problem, he said, is to build ships capable of following the icebreakers through the paths they have cleared.

Meanwhile, the world's first research icebreaker, the 'Academician Otto Schmidt' is being built in Leningrad for the Institute of Arctic and Antarctic Research. The vessel will carry fourteen laboratories for oceanological, meteorological, hydrological, and ice research, a special unit for aqualung divers and a computer for the on-board processing of research results.

● Using 'Pisces-7' and 'Pisces-11' self-propelled diving craft, a limnological expedition has succeeded in reaching the bottom of Lake Baikal, the biggest and deepest rift-trench in Asia. According to Evgenii Mirlin, leader of the expedition, the main purpose was to apply the techniques developed for ocean-bed research to a study of the lake bed. The programme includes underwater photography of geological formations, and a study of the unique flora and fauna of the lake. Even at the greatest depths, said Mirlin, shrimps, Baikal bullheads, and the small semitransparent *Comphorus baicalensis* can be found.

The team spoke highly of the 'Pisces' craft, which, in addition to providing facilities for visual observations and still and TV photography, have special mechanical arms for collecting geological samples and specimens of water.

Vera Rich

IN BRIEF

A voice for geologists

Geologists in Britain who have felt for some time that they should have a collective voice for the profession have just founded an Institution of Geologists. The institution will aim to advance the profession and practice of geology by 'shaping opinion' in various centres of power and taking an interest in matters of professional standards, education, remuneration and so on. There are about 4,000 geologists in Britain, and a quarter of them have taken an interest in the founding of the institution.

The Geological Society of London, which will provide premises for the institution, was unable to perform these functions itself by virtue of being a learned society with charitable status. But in the long run many hope that the two bodies will operate jointly.

New line of questioning

The case of the dissident Moscow mathematician, Anatolii Shcharanskii, who has been held incommunicado since his arrest last March has now taken an ominous turn; last week, his fellow refusenik Vladimir Lazaris was called to the Lefortovo prison to answer questions about Shcharanskii's mental stability. Although Moscow activists feel it is too early to assess the significance of this new line of questioning, they feel that its proximity to the International Psychiatric Congress in Honolulu, at which the Soviet Union was censured for its use of psychiatric means of political repression, should not be ignored.

CFC policy

The UK government's policy not to ban the use of chlorofluorocarbons

(CFCs) in aerosols until much more research has been undertaken, is supported in a statement issued last week by the Clean Air Council, a statutory body set up to advise the Secretary of State for the Environment on air pollution. The Clean Air Council regards as tenuous the argument that CFCs lead to depletion of the ozone layer in the atmosphere allowing increased ultraviolet radiation to reach earth and possibly cause damage to health (for example, skin cancer).

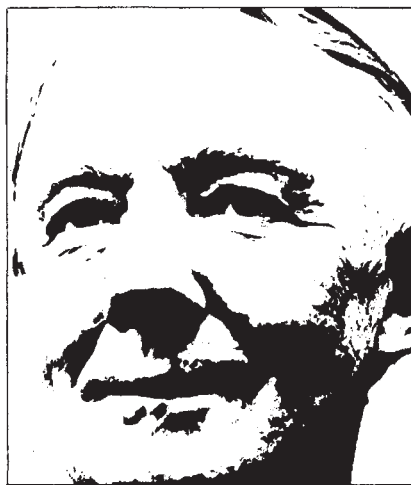
Since measurements began in the 1920s, the amount of ozone has in fact increased by 5-10%. The statement points out that 'natural' variations are enormously greater than those so far calculated to be a result of the use of CFCs. While supporting further research on CFCs, the Council regards the moves in the USA to ban their use as "precipitate".

THE World Health Organisation (WHO) is rightly concerned with nutrition, and with the more efficient production and distribution of the earth's resources. WHO has fortunately stopped putting forward absurd statements, such as the one that more than half the world's population is starving, which damaged its earlier reputation. The situation in many countries is serious enough without dramatic exaggerations. Some millions of people die annually of starvation, and several hundreds of millions are underfed for at least a part of the year.

Yet there is no need for this suffering. The world produces more than enough for every member of its population to receive an adequate and healthy diet. If waste and greed could be abolished, there would already be enough to feed the extra three or four thousand million mouths expected by the year 2,000. People starve, or are underfed, because they are poor, and cannot compete in the world market with the consumers in rich countries. The most voracious and wasteful of these consumers are livestock, the battery hens, pigs in sweat houses and barley beef, which waste both calories and protein to add to the pleasure of western palates.

The richer countries also have nutritional problems, but these arise from over and not under eating. When we hear that one of our friends is 'on a diet' we immediately understand that he is trying to lose weight gained by previous over-indulgence. Slimming is becoming big business. A substantial part of the food processing industry is making

allegedly non-fattening products. Newspapers fill their columns with bizarre diets which are often so revolting that their victims will lose weight. Health farms charge over-fed

Cuisine minceur**KENNETH MELLANBY**

pop stars more than five star hotels for organised starvation. Even reputable scientists explain how we may try to mislead our stomachs into believing that they have been generously indulged on a minimum number of calories.

A sophisticated attempt at making the best of both worlds—of gluttony and abstinence—is made by Michel Guérard in his new *Cuisine minceur* roughly translated as 'Slimming cookery'. The author runs one of the best, and most expensive, restaurants

in France. His book claims to be "an authoritative and authentic guide to a revolutionary new method which is sweeping the world of haute cuisine". It contains some good recipes; I was glad to learn how to produce a delicious *purée-mousse d'artichauts* and use up the glut of globe artichokes, one of the few vegetables spared by the plague of rabbits in my Huntingdonshire garden. But the new method seems mainly to eschew the more calorific dishes, and to use sugar substitute and *crème fraîche*, a tiresome preparation containing buttermilk. In fact in her introduction, Shirley Conran gives the game away, pointing out that M. Guérard's portions are small, for "if you eat large quantities of food, no matter what it is, you are not going to lose weight". Surely it would be sensible to avoid all this bother, and eat even smaller amounts of normal recipes. M. Guérard's dishes taste even better when made with real cream.

Clearly we should make the best of both worlds. If we in the West ate less we should be healthier, and there would be enough for the underfed nations; surely mankind is not too stupid to be unable to solve the economic difficulties. But we must learn that the only cure for over-eating is abstinence, not a fancy diet. Few are as naïve as the plump girl I heard lamenting her inability to eat enough starch-reduced bread, but the view that some foods encourage thinness is still widely held. High-protein diets are only slimming because they are so expensive. We should remember that the only sweetmeat with a truly non-fattening centre is the polo mint.

correspondence

Roots of 'enzyme'

SIR,—There can be no doubt that Kühne introduced the word *Enzym*, from which, of course, was formed its English equivalent. The reasons for his choice are discussed in at least two printed sources^{1,2}. But the actual construction of the word from Greek originals is less certain, in spite of some very definite statements in the literature.

Bayliss gave a translation of the relevant passage of reference 2, in two slightly different versions in *The Nature of Enzyme Action*, page 11–12 (Longmans, London 1925) and *Principles of General Physiology* (Longmans, London). The words concerned in the former are as follows “... but it merely states that *εν ξυμη* [in yeast] something occurs that exerts this or that activity, which is considered to belong to the class called fermentative”. Nothing could be clearer: rarely do we have as precise knowledge as this of the thought processes of an author. However, Greek words do not occur in the original at all. They are an interpolation by the translator, whose immense authority and influence has led to almost universal acceptance of this version. The original runs at this point, “... sondern nur gesagt, dass in der Zyme etwas vorkomme, das diese oder jene zu den fermentativen gerechnete Wirkung habe ...”.

Bayliss's translation of this passage is, in other respects also, remarkably inaccurate. But unless there is concrete evidence of Kühne's intentions we are reduced to considering probabilities, and this direct construction from *εν ξυμη* still seems rather more likely than the alternative etymology put forward in *The Oxford English Dictionary*. According to this authority, the word was formed on modern Greek *ενξυμος*, meaning 'leavened', or more specifically the leavened bread used in the Eucharist of the Greek Orthodox Church. The very word 'enzyme' was used in English in 1850 in this sense. It seems unlikely that Kühne would have had any knowledge of modern Greek and, if not, this derivation appears to be impossible, even though it preserves a connection with the primitive meaning of the word 'ferment' which Kühne was seeking to replace.

As pointed out by Plantefol, in

Comptes Rendus de l'Académie des Sciences 226C, 1976, a compound had already been formed in Greek meaning 'yeast in' rather than 'in yeast', so that Kühne's probable sequence of thought was somewhat unfortunate. But even Plantefol seems unaware of Bayliss's blunder. Plantefol also quite justly comments that 'enzyme' has merely taken over the functions of the pre-existing word 'diastase'. (In neither case is the etymology of any relevance to modern ideas of the nature and function of the substances concerned). It seems regrettable, if irretrievable, that this usurpation should have occurred.

Yours faithfully,

T. R. C. BOYDE

Department of Biochemistry,
University of Hong Kong,
Hong Kong

- 1a *Verhandlungen des Naturhistorisch-Medizinisch Vereins zu Heidelberg* 1 Pp 190–3 (1877). Formally anonymous report of a conference speech by Kühne. Recently reprinted with reference 1b in facsimile in *FEBS Letters*, supplement to 62 (1976).
- 1b Kühne, W. *Verhandlungen des Naturhistorisch-Medizinisch Vereins zu Heidelberg* 1 Pp 194–8 (1877).
- 2 Kühne, W. *Untersuchungen der physiologischen Institut der Universität Heidelberg* 1 Pp 291–324 (1878).

Proton-electron mass ratio

SIR,—Sirag (28 July, page 294) states that the proton-electron mass ratio is 1836.109, and derives a combinatorial prediction of 1836 for this quantity. The most recent editions of the *Review of Particle Properties* give 1836.1514 ± 0.0073 , some 21 standard deviations away. While the accepted value for the proton mass has changed significantly since about 1973, the value 1836 for the ratio m_p/m_e has been getting less and less plausible for at least the past 15 years.

The value $6\pi^5 = 1836.1181$ has been calculated, on very different grounds, to be this mass ratio (see *Physics Today*, page 17–19, August, 1971, for discussion). It is substantially closer to the most recent experimental value, but is still in disagreement by 4.6 standard deviations. It is also perhaps worth drawing attention to the apparent tendency of stable hadrons (and the muon) to have masses which are integral multiples of $3m_e$, the threefold electron mass. This has been discussed by R. Frosch in *Lett. Nuovo Cim.* 8, 633 (1973), and at a meeting of the

Swiss Physical Society, Geneva, 8–9 October, 1976.

Yours faithfully,

JOHN F. CRAWFORD

SIN, Switzerland

Was Darwin the Bellman

SIR,—The uncanny resemblance of F. Waddy's engraving of Darwin on the cover of 21 July to Henry Holliday's picture of the Bellman in the 1876 edition of *The Hunting of the Snark* by Lewis Carroll (Macmillan) (see below) and the 1962 edition of *The Annotated Snark* by Martin Gardner (Penguin Books), must have caused many to suspect that Darwin was the Bellman. This suspicion is confirmed by the two tufts (of hair?) at the foot of Waddy's picture, strongly suggesting that Darwin, in

“Supporting each man on the top of the tide

By a finger entwined in his hair”
had inadvertently scalped two of the crew.

Was it really the Snark that the Bellman was after in his voyages in H.M.S. Beagle (see pp28–31 and p52 footnote 17 in *The Annotated Snark*)?

Yours faithfully,

H. J. A. DARTNALL

MRC Vision Unit, University of
Sussex,
Brighton, UK



news and views

Ultraviolet observation of a quasar spectrum

from B. E. J. Pagel

QUASARS of high redshift permit emission and absorption lines of the far ultraviolet region to be observed from the ground. But what would the spectrum of a quasar look like if it could be observed over a really wide range of (rest) wavelength, say from 0.09 to 1 μm ? At first sight one might think that this question can be answered by putting together the spectra of several quasars spanning the full range of observed redshifts, and indeed a good deal of useful work has been done along these lines leading in particular to the discovery that the Lyman- α emission line of hydrogen is not much stronger than the red H α line although straightforward theories predict that it should be about an order of magnitude stronger. However, there are severe difficulties in piecing together the spectra of quasars with different redshifts because of the wide range in their intrinsic properties: thus (if one makes the most plausible assumption, that the redshifts are 'cosmological', that is, symptomatic of great distances) the quasars that are actually observed generally become intrinsically more luminous at the higher redshifts, their brightnesses are subject to uncertain cosmological corrections and they are seen through increasing amounts of intervening material in intergalactic space and possibly in clusters of galaxies and even in galaxies themselves.

Consequently it has been clear for some time that observations of the ultraviolet spectra of quasars would help to answer crucial questions concerning both the nature of quasars and the distribution of diffuse matter in the Universe. Furthermore, if 'standard candles' can somehow be identified among quasars, then there is the possibility of using the enormous distances to which they can be seen to obtain really decisive results in

cosmology. But quasars are faint (the brightest one by far being 3C 273 with apparent magnitude 13 and redshift 0.16) and have been beyond the range of past experiments with rockets and ultraviolet satellites, so that the first results were expected to come from the next generation of space observatories using large reflectors, particularly the IUE (to be launched at the end of this year) and the NASA Space Telescope (planned for 1983).

Some of the exciting results awaited from the next generation of space telescopes have now been anticipated, however, in a remarkable rocket experiment carried out by members of the Physics Department of Johns Hopkins University (Davidsen, Hartig and Fastie, this issue of *Nature*, page 203), in which a Faint Object Telescope (FOT) of 40 cm diameter (the largest ever to be flown in a sounding rocket) with a concave grating giving a resolving power of about 150 was used to observe the spectrum of 3C 273 between 1,080 and 1,465 $\text{\AA}$ in its rest frame.

Despite the limited resolution, Davidsen *et al.* have been able to draw a number of interesting conclusions from their spectrum of 3C 273. It shows Lyman- α and other strong emission lines seen in high redshift quasars and confirms the low ratio of Lyman- α to H α which may be due to dust in the line-emitting region or some other causes. The flux at the Lyman continuum limit (important as an input datum for photoionisation models of the line-emitting region) can be estimated from a small extrapolation of the observed continuum, and the absence of an absorption trough on the short side of Lyman- α enables the previous very stringent limit on the abundance of neutral hydrogen in intergalactic space to be extended to recent cosmological epochs.

A traditional preoccupation in extragalactic astronomy is the determination of the deceleration parameter q_0 , the basis of the method being to plot the redshifts of 'standard candles' against

their apparent brightness so that deceleration manifests itself as an upward curvature in the relation at the faint end. The favoured choice for standard candles is to use first-ranked elliptical galaxies in clusters, but these are restricted to redshifts less than about 0.5 and raise the problem of the effect on their luminosities of stellar evolution during the look-back time; so all that one can say with confidence from present data is that q_0 is fairly small, between -1 and $+1$, say. Indirect arguments, based on the large cosmic abundance of deuterium, suggest that q_0 is positive and under 0.1, well below 0.5 which is the boundary between a 'closed' and 'open' Universe.

Recent work on high redshift quasars has suggested that there may, in fact, be a method of finding standard candles among quasars, based on the equivalent width of Lyman- α , and perhaps one or two other emission lines, in those objects which are flat-spectrum radio sources. Since 3C 273 is a member of this class, Davidsen *et al.* have been able to use their measurement of Lyman- α to tie it in to the system of standard candles based on objects with redshifts up to 3.39 and thus to deduce that $q_0 = 1$ with an impressive internal precision, but with a possibility of systematic error which the authors are careful to point out. A further point that might be made is that the fact of their getting a reasonable value of q_0 at all by this method is evidence in favour of the cosmological interpretation of the redshift.

Probably the most significant result of all in the Johns Hopkins experiment relates to the nature of the absorption lines seen in quasars of high redshift. These fall into two classes: lines seen close to (and usually on the blueward side of) the emission lines, which can be very broad and are almost certainly a 'P Cygni' effect of absorbing gas coming out from the quasar itself (Class I); and Class II absorption lines (including occasionally the 21-cm line of neutral hydrogen), seen at much

lower redshifts than the emission lines, which are narrow, are formed under low-excitation conditions and can exist in many different redshift systems in the spectrum of one object. The nature of the Class II absorption lines is highly controversial. Rival hypotheses state on the one hand that they are due to material ejected from the quasar at speeds up to $0.6c$; and on the other that they are due to diffuse interstellar gas in unseen intervening galaxies at the normal cosmological redshift. The second hypothesis is favoured by the distribution statistics of number of systems in one object and by the resemblance between the absorption-line spectra and that of the interstellar medium in our own neighbourhood, but requires the extent of interstellar gas in at least some galaxies to be larger than expected. The presence or absence of Class II absorption lines (which are chiefly

seen shortward of Lyman- α) in 3C 273 provides a critical test between these two hypotheses, because 3C 273 is as luminous as at least one of the high redshift quasars in which several absorption-line systems are seen and is presumably very similar to them physically so that it is equally capable of expelling matter; but it is so near to us that the probability of an intervening galaxy being present is negligible.

Although the spectral resolution of the experiment of Davidsen *et al.* is too low to detect individual absorption lines, the number of lines in high redshift objects is so great as to produce a depression of about 30% in the continuum below Lyman- α , and this can still be easily detected at low resolution. The observed depression in 3C 273 is only $2 \pm 6\%$, which provides an impressive argument in favour of the intervening-galaxy hypothesis. $\square$

Cell transformation with herpes simplex viruses

from Franklin H. Portugal

HERPES simplex virus, one type of which causes cold sores and another genital tract infections, is receiving increasing attention as a model for understanding how virus expression leads to host cell transformation. In certain conditions infection of mammalian cells with HSV leads to a non-productive infection in which only part of the viral genome is expressed. When mouse cells lacking the enzyme thymidine kinase are transformed by ultraviolet-irradiated HSV, the cells develop thymidine kinase activity which is coded by the virus and which can be distinguished from the host cell enzyme by various biochemical criteria. So far cells transformed in this way cannot be induced to release infectious virus, suggesting that a fragment of the viral genome, although unable to produce infectious virus, is able to maintain the virus-transformed phenotype. Nucleic acid hybridisation indicates that in mouse cells infected with irradiated HSV-1, either five copies of about 23% of the viral genome (Kraiselburd, Gage & Weissbach *J. molec. Biol.* **97**, 533; 1975) or four to six copies of a 10% fragment of the viral genome (Davis & Kingsbury *J. Virol.* **17**, 788; 1976) are present. Hamster cells transformed by irradiated HSV-2 seem to

contain one to three copies of between 8% and 32% of the viral genome (Frenkel *et al. J. Virol.* **18**, 885; 1976). It is not yet known whether the viral genes become integrated into the host chromosome or remain in the cytoplasm.

Maitland and McDougall (*Cell* **11**, 233; 1977) now report that they have been able to transform mouse cells lacking thymidine kinase activity with DNA fragments produced from HSV-2 DNA either by specific cleavage with restriction endonucleases or by mechanical shearing. The transformed cells were selected by their ability to grow in medium containing hypoxanthine, aminopterin, and thymidine, which indicates the presence of a thymidine kinase-positive cell. The cells contained the virus-induced enzyme as well as HSV-specific immunofluorescence. The DNA fragments produced by endonuclease cleavage can be located on a map of the HSV-2 genome and from this information, the thymidine kinase gene seems to be located in the long segment (L region) of the viral genome. Wigber *et al. (Cell* **11**, 223; 1977) report the transformation of thymidine kinase-negative mouse L cells with a *Bam*I endonuclease restriction fragment of HSV-1. Cells treated with *Eco*I restriction fragments were not transformed. Bacchetti and Graham (*Proc. natn. Acad. Sci.*

U.S.A. **74**, 1590; 1977) also report that thymidine kinase-negative human cells can be transformed by mechanically sheared fragments of HSV-2 DNA. Several lines of transformed cells have been established that grow continually in the selection medium, express thymidine kinase activity of viral origin, and differ in the stability of thymidine kinase expression.

As well as thymidine kinase, a recent study suggests that a new species of another enzyme, deoxycytidine deaminase, is induced when mammalian cells are lytically infected with HSV-1 (Chan *Proc. natn. Acad. Sci. U.S.A.* **74**, 1734; 1977). The results strongly suggest that this enzyme is also coded by the virus. This study is of particular importance because the induced enzyme may affect cytosine arabinoside, a drug commonly used to treat HSV infections, thus making it less effective.

The fact that specific fragments of the HSV genome, coding for clearly defined biochemical functions, can transform mammalian cells suggests that the HSV system will become an increasingly important model for defining those genes required for both biochemical and morphological transformation. In addition, this system has already begun to provide evidence for the mechanisms that control the viral genome in mammalian cells. This information will presumably be of use not only for specific chemotherapy of human HSV infections but also for understanding virus transformation in general, including oncogenic transformation. Furthermore, it may eventually become feasible to use the HSV model for DNA-mediated gene transfer between mammalian cells. $\square$

Electron storage rings as lasers

from John Pendry

A NEW mechanism for laser action has been demonstrated by a research group working at the Stanford linear accelerator (Deacon *et al. Phys. Rev. Lett.* **36**, 892; 1977). The device is built on the theory that an electron deflected by a magnetic field can emit stimulated as well as incoherent radiation. So far the device has worked in a pulsed mode extracting 7 kW peak power of laser radiation from the kinetic energy of the electron beam. It appears that only

John Pendry is Head of the Theoretical Physics Group at the Daresbury Laboratory.

about 0.2% of the electron's kinetic energy can be extracted in each pass through the magnets but it is planned to increase the efficiency by circulating the electrons in a storage ring, which should also raise the mean power available.

Conventional electron storage rings have a large amount of power pumped into them which is dissipated in the incoherent synchrotron radiation. Ultimately this new laser, fixed to a storage ring, could conceivably channel a substantial fraction of the power into stimulated radiation making it available as a well collimated beam of fixed wavelength.

In a conventional laser transitions are stimulated between electronic states of atoms. The power is limited by the pumping process for energising the atoms, and the frequency by availability of suitable electronic transitions. Only recently have continuously tunable lasers become available and the range of tuning is limited to typically less than 1% of the operating wavelength. In the new process massive amounts of energy are available in the kinetic energy of the electron beam and the wavelength of radiation can be simply varied by a large factor. It is expected that lasing action can be observed at wavelengths varying from a few 100 μm down to 0.1 μm .

The laser operates by passing the electron beam through a helical magnetic field. If the spontaneous radiation is confined in a cavity, it can stimulate further radiation, the gain peaking at a wavelength

$$\lambda_s = \frac{\lambda_q}{2\gamma^2} \left[1 + \frac{1}{(2\pi)^2} \frac{\lambda_q^2 r_0 B^2}{mc^2} \right]$$

where λ_q is the period of the helix, B the magnetic field strength, c the velocity of light, r_0 and m the classical electron radius and mass of the electron respectively; the electron energy is γmc^2 . The electron chases closely after the emitted radiation with almost the velocity of light so that pulses from successive periods of the helix fall very close together and the wavelength of radiation is much shorter than the helix period. A 3.2-cm period helix and 43.5 MeV electron beam generate 3.4 μm radiation. (The helix used in the experiment was 5.2 m long.) It is this relativistic shift of the wavelength which gives the laser its great tunability. The energy of the electron beam being proportional to γ , the operating wavelength, λ_s , scales inversely as the square of the energy.

The immediate difficulty is that the laser requires high currents to produce usable gain, posing problems of stability in storage rings operating at what are

rather low electron energies. The theory of storage rings is well understood and these difficulties can probably be overcome to a satisfactory extent. Less well understood is the perturbation that the huge radiation fields building up in the cavity will have on the electron orbits. Even in these preliminary experiments $\frac{1}{2}$ MW of peak power was present in the cavity and this amount of radiation could possibly upset the operation of a storage ring.

Devices producing large amounts of power over a wide spectral range are bound to find wide application. It is conceivable that one of the first uses might be as a tunable source of infrared radiation. Current sources tunable in the 20–200 μm range are of very low power, 10 mW or less, whereas the free electron laser has already functioned at an average power of 0.36 W. Spectroscopy in the far infrared would be vastly improved by the availability of even 1 W of power. Experiments on semiconductors would have the sensitivity to measure excitonic and impurity spectra. Many transient species occurring in fast reactions or in the stratosphere would become accessible to laboratory infrared spectroscopy, and infrared studies of surfaces would be much extended in their capabilities if the weaker absorbers of radiation could be detected.

If the free electron laser can be further developed to give very high powers approaching the megawatt c.w. range sufficient power would be available to consider photochemical reactions and isotopic separation on an industrial scale. In the laboratory large amounts of radiation could be used to shift populations of states on a macroscopic scale giving rise to non-linear phenomena. But it is probably true that the most valuable uses of such a source are yet to be discovered. $\square$

Planetary crater retention ages

from David W. Hughes

ABSOLUTE ages of planetary features can be determined in principle by dividing the observed crater number density N by the crater production rate, F . So age = N/F . William K. Hartmann (Planetary Science Institute, Tucson, Arizona) is of the opinion that 'the emphasis in funding, research and publication is all on the numerator and none on the denominator' in the above

David W. Hughes is a Lecturer in the Department of Physics at the University of Sheffield.

Correction

In the article 'Multispecific antibodies' (*News and Views* 268, 689; 1977) column 2, page 690, paragraph 2, line 4 should read: "The 'best' antibodies bind their antigens with avidities of 10^7 – 10^9 1 mol^{-1} but so far multispecificity has only been observed in the $\leq 10^6$ 1 mol^{-1} range."

equation. He aims to remedy this state of affairs by his recent paper in *Icarus* (31, 260; 1977).

First let us deal briefly with the numerator. The literature abounds with papers giving details of how N varies with diameter D . Researchers have obtained photographs of lunar and planetary features from Orbiter, Surveyor, Apollo and Mariner spacecraft and have simply counted N , the number of craters per square kilometre with diameters in the range D to $\sqrt{D}$ km. N is proportional to D^n where $n = -2.0$ for lunar maria and the Tharsis plane of Mars, regions where there has been little disturbance. Erosion, which removes smaller craters, gives n values between -1.3 and -1.8 . On the slopes of Olympus Mons, the Martian volcano, $n = -2.4$, possibly due to small volcanic pits adding to the impact craters. To give some impressions of the numbers involved, a typical lunar maria which has been exposed to impact bombardment for about 3.4 aeons (an aeon is 10^9 yr) would have about 10,000 craters larger than 1 km in diameter and 10^{13} larger than 10 cm, in an area of 10^6 km².

Hartmann deals with F , the crater production rate in considerable detail. F is a function of crater diameter, time from the origin of the Solar System, heliocentric distance and planetary size. The diameter dependence is fairly well understood and a similar power law dependence has been found for crater populations on young undisturbed plains on Mercury, Mars and the Moon. The dependence is consistent with the power law size distribution of asteroids, comets and meteorites, these being the impacting bodies responsible for the craters.

The time dependence is based on Apollo chronologies. F was at least hundreds of times higher than its present value before 4 aeons ago but has been within a factor of 10 of present day values during the past 3 aeons. Most people regard the time dependence as representing a smooth transition from the chaotic state of affairs during planetary accretion to the present day near steady state depletion of the asteroid and comet reservoirs. F would be expected to have roughly the same time dependence for all planets.

The change in F as one goes from one planet to another is virtually unknown and is the primary subject of Hartmann's paper. He bases his calculations on a study made by G. Wetherill (*Proc. Lunar Sci. Conf.* 6, 1539; 1975) who considered families of planetisimals in specific orbits and then proceeded to compute the relative number of impacts from each family onto the surfaces of the different planets. The first step is to consider a meteorite of a specific mass and to calculate the diameter of the impact crater it would produce on Mercury, Venus, Earth, Moon, Mars and so on. Obviously the meteorite's orbital velocity (and thus energy) will increase as it moves in towards its perihelion. The effect that the planetary gravitational attraction has on this velocity also varies (this is proportional to $\sqrt{M/R}$ where the planet has a mass M and a radius R). The more massive planets also have a larger effective collision cross section and can sweep up more impacting bodies. Four orbital families are considered, ancient planetisimals circling planets (these only hit planetary satellites), ancient planetisimals circling the Sun (these two families having low relative velocities), asteroidal and short period cometary bodies making up a medium velocity family and long period comets, a high velocity family. In the case of the Moon the impact velocity of a typical member of each of these families would be about 3, 6, 14 and 38 km s⁻¹ respectively. The calculated F values from each source onto each planetary and satellite body turn out to be remarkably similar and Hartmann concludes that the terrestrial planets have crater production rates within a factor of 10 of each other. No planet seems to have a preferential source of impacting bodies. The Mercurian crater production rate is about 0.8 to 5 times the lunar rate indicating that Mercury's lava plains, which have N values equal to their lunar counterparts, are in fact younger. Venusian and terrestrial cratering rates are about 1 to 2 times the lunar rate, the surface of Venus having a retention age for 100 km scale craters of around an aeon. Martian production rates are between 1 and 7 times higher than lunar rates; more of this later. The satellites of the outer planets have crater production rates less than a tenth the rate of lunar cratering and should be considerably smoother. There seems to have been no major fluctuations in these rates over the past 3 aeons, and Hartmann concludes that the planetary crater retention ages of surface features formed during this time can be determined within an uncertainty factor of 3 in either direction.

The calculations discussed above have been applied specifically to

Martian crater counts. By scaling to a typical lunar maria the age of a Martian feature, T , is given by

$$T = 3.4 (N_{\delta} / N_{\circ}) (F_{\circ} / F_{\delta}) \text{ aeons}$$

The channels on Mars are found to have ages of 1 aeon (the uncertainty factor making this range between 0.3 and 3 aeons). The Tharsis plain, a broad equatorial region which surrounds four volcanoes, is slightly younger, about 0.8 aeons old. Olympus Mons is about 0.2 aeons old. These latter findings are contrary to recent assertions by G. Neukum and D. U. Wise (*Science* 194, 1381; 1976) who find that Olympus Mons is 2.5 aeons old and most Martian volcanic provinces older than 3 aeons. Hartmann puts forward a hypothetical Martian scenario in which liquid water was much more abundant on Mars before the Tharsis volcanism started. This volcanism caused a variation in the obliquity of Mars which switched greenhouse effects on and off forming and remelting permafrost layers. This continued until the current Tharsis surface was formed which fixed the obliquity at its present value and resulted in the present state of nearly permanent cold dryness.

Two things are needed to refine the study of crater production rates. First the number of velocity and orbital families considered must be increased and statistical calculations must be made to give a more accurate estimate of the final impact site of bodies in these families. Second, more asteroidal and cometary surveys must be undertaken so that more accurate estimates can be made of the numbers of bodies in each orbital family. With this knowledge the uncertainty factor of 3 in the crater retention ages can one hopes be much reduced. □

Irrepressible superconductor

from Robert W. Cahn

THOSE who study superconductors with practical ends in mind have been much concerned to raise the transition temperature and the critical magnetic field: indeed, one sometimes has the impression that a transition temperature at or above ambient represents the philosopher's stone for some of today's physicists. There is however another, less regarded material property which limits potential applications, and that is the critical current density, itself a function of field and a structure-

sensitive property. Nb₃Al is one of the most promising A-15 superconductors because of its extremely high critical field (H_{c2} = 295 kG at 4.2 K), but the critical current density is modest, and various metallurgical tricks used by a number of investigators to modify the microstructure have in the past not been very effective in improving the situation. About 10⁵ A cm⁻² is the best that has been achieved. A completely new approach now promises the engineer an excellent new superconductor.

K. Lo, J. Bevk and D. Turnbull (*J. appl. Phys.* 48, 2957; 1977) of Harvard University decided to melt-spin the alloy. 'Melt-spinning' is the name given to a variant of splat-quenching which produces a thin continuous ribbon of alloy. The version used here involves a rapidly rotating copper saucer—the substrate—on to which a fine stream of molten alloy is projected. The investigators found that a small silicon addition was needed to produce a good ribbon: their alloy was of composition Nb₇₃Al_{27.5}Si_{1.5}. Hitherto, melt-spun ribbons have been used to examine mechanical or ferromagnetic behaviour: melt-spun superconductors are something new under the Sun.

The thickness of the ribbon and thus the effective quenching rate can be modified by altering the speed of rotation of the substrate: the thinnest ribbon was 25 μm in thickness and had grains <0.5 μm in diameter. The microstructure consisted of fragments of dendrites surrounded by transition layers presumed to be of variable composition. The crystal structure remained single phase A-15. To measure electrical characteristics, the samples had to be embedded in indium in order to provide good electrical and thermal connections. Even so, the contact resistance limited the feasible current density to 1.8 × 10⁶ A cm⁻². 25 μm ribbons were tested in fields up to 150 kG and the critical current density was found to be in excess of the experimentally enforced limit of 1.8 × 10⁶ A cm⁻² in all field strengths. When the ribbon was annealed at 750 °C, T_c was raised from ~16 K to 18.4 K and the critical current density was still in excess of the experimental limit. (It is therefore not known whether heat treatment increased or decreased the critical current density). The highest critical current density in 105 kG for Nb₃Al (that is, the highest in any substance) reported in the past was ~10⁵ A cm⁻². Plainly an extreme refinement of microstructure is the key to an enhanced critical current density.

It is already clear, from these preliminary results with just one of the A-15 family of compounds, that a new generation of high-field super-

Robert W. Cahn is Professor of Materials Science at the University of Sussex.

conductors is in the offing. There will of course be problems—principally the need to incorporate superconducting ribbons into a thermally well-conducting matrix such as copper to provide insurance against a breakdown of superconductivity in service. A number of metallurgical techniques are available for this purpose (for example, Tsuei *J. appl. Phys.* **45**, 1385; 1974; Chen & Tsuei *ibid.* **47**, 715; 1976) but they generally depend on a precipitation of superconducting filaments *in situ*. Melt-spun ribbons will have to be incorporated in a matrix after they have been made: it may be possible to use a sintering technique with a low-melting metal such as aluminium. The fact that annealing does not damage the superconducting properties will simplify the task.

Rapid quenching from the melt has been used before in connection with superconduction, but in a different sense. Instead of using the technique to refine the structure of a stable compound, as the Harvard scientists have

done, workers at California Institute of Technology used it to create metastable structures. Johnson and Poon (*J. appl. Phys.* **45**, 3683; 1974) tested a whole range of metastable simple cubic intermetallic superconductors (such as $\text{Te}_{70}\text{Au}_{30}$, $\text{Sb}_{75}\text{In}_{25}$) and established that T_c was systematically (and inversely) related to lattice parameter; $\text{Sb}_{75}\text{Au}_{25}$ had the highest T_c of ~ 6.5 K, and it seemed that the (meta) stability of the simple cubic lattice (one atom per unit cell) broke down for smaller lattices. Glassy alloys, made by melt-spinning, can also be superconducting: V. L. Johnson, S. J. Poon and P. Duwez (*Phys. Rev.* **B11**, 150; 1975) examined the superconductivity of $\text{Au}_x\text{La}_{100-x}$ glasses ($16 \leq x \leq 40$). Among other peculiarities, these glasses have ultra-short electronic mean free paths. For $\text{Au}_{22}\text{La}_{78}$, this path is $1.7 \text{ \AA}$, $T_c = 3.4$ K, the upper critical field ~ 10 kG. This category of material is a scientific curiosity but is not likely to threaten the technological superiority of Nb_3Al and its analogues. $\square$

tion of heat sources is uncertain and must be assumed, albeit on the basis of reasoned geological and petrological arguments. In practice, Chapman and Pollack take an upper layer of 8 km with heat production A overlying a granulite facies lower crust of 32 km with heat production $0.25 \mu\text{W m}^{-3}$ overlying a depleted ultrabasic zone (80 km, $0.01 \mu\text{W m}^{-3}$) overlying a pyrolyte mantle ($0.084 \mu\text{W m}^{-3}$). In this context, A , the heat production in the upper crust, is the variable related to surface heat flow. By inserting different values of regional surface heat flow it is then possible to derive a series of geotherms for the continental lithosphere (making another assumption, again reasoned, on the variation of thermal conductivity with temperature).

The approach to the oceanic area, however, is rather different. Here the thermal regime is characterised by cooling following formation at a spreading ridge, although the oceanic lithosphere will also contain radioactive sources. Chapman and Pollack assume an initial temperature gradient of $1,300^\circ\text{C} + 1.5^\circ\text{C km}^{-1}$ and a heat production of $0.5 \mu\text{W m}^{-3}$ in the upper 10 km in deriving a family of oceanic geotherms in which the variable is again regional heat flow. And finally there is the question of the mantle solidus, for which assumptions are again necessary because of the uncertain role of volatiles in depressing solidus temperature. In fact, Chapman and Pollack go for a mixed volatile environment (chiefly H_2O and CO_2) which leads to a solidus intermediate between those applicable to volatile-free and purely hydrous melting.

Implicit in all this, of course, is the hope of an ultimate relationship between (regional) surface heat flow and lithospheric thickness which can be defined accurately and smoothly enough to enable the latter to be determined from the former. And such a relationship indeed emerges. From the intersections of the derived geotherms with the adopted solidus, Chapman and Pollack are able to obtain smooth curves (one each for continental and oceanic zones) of lithospheric thickness against heat flow. Moreover, where comparable direct data on lithospheric thickness are available (for example, for the Pacific and North America) the agreement between observation and the predicted curves is good, thus justifying the assumptions made in the first place. With this encouragement it is then but a short step to convert the world map of the 12th degree spherical harmonic analysis of global heat flow developed earlier by Chapman and Pollack (*Earth planet. Sci. Lett.* **28**, 23; 1975) into a parallel map of world lithospheric thickness.

Thickness of the lithosphere

from Peter J. Smith

FOR more than 50 years the Moho was regarded as the most important discontinuity in the upper Earth, and following its discovery by Andrija Mohorovičić in 1909 considerable effort was devoted to determining its depth throughout the world. The result is that worldwide contour maps, albeit imperfect ones, of crustal thickness have been around now for some time. But although the Moho is no less fundamental a boundary than it ever was, its significance has been overshadowed in recent years by that of the lithosphere–asthenosphere interface. The fact is that as far as plate tectonic processes are concerned, the crucial contrast is not the physicochemical one between the crust and mantle but the physical one between the lithosphere and asthenosphere.

So how thick is the lithosphere? A rapid and random survey of published opinions suggests that the top of the asthenosphere is most commonly thought to be 'about 70 km deep', although it is widely recognised that this is an average concealing variations. In this latter respect lithospheric thickness resembles crustal thickness; but the similarity ends there. The lithosphere–asthenosphere boundary appears to be less sharp than the Moho and thus more difficult to locate

accurately. Moreover, there are far fewer spot measurements of asthenospheric depth than of mantle depth because of the relatively recent origin of interest in the former. Certainly no one has yet suggested that there are sufficient direct data for the production of a worldwide contour map of lithospheric thickness.

Nevertheless, Chapman and Pollack (*Geology*, **5**, 265; 1977) have now produced just such a map. Of course, their approach has had to be indirect. Specifically, they have developed a thermal model of the lithosphere which has enabled them to derive a family of geotherms for continental and oceanic zones in which the principal independent variable is surface heat flow. The depth at which these geotherms intersect the mantle solidus is then taken as the surface of the asthenosphere. The point about all this is, of course, that surface heat flow and its variations throughout the world are now quite well known, so the problem of the primary data is largely solved.

On the other hand, there is a disadvantage in that, as with all indirect methods, it is necessary to make assumptions. For example, the continental model adopted by Chapman and Pollack not surprisingly involves steady state conduction through a lithosphere which contains radioactive heat sources and into which heat flows from below. But the vertical distribu-

What the new map shows is that the thickness of the lithosphere varies from a few tens of kilometres in young oceans and continental orogenic provinces to more than 300 km in shield areas. It is also evident from the analysis which led to the production of the map that beneath the continental shields the asthenosphere is very thin. Indeed, a strict interpretation would suggest that the asthenosphere does not exist there at all, for shield geotherms do not intersect the solidus at any depth, although shield lithosphere is presumably partially decoupled from the deeper interior at the depth at which the appropriate geotherm approaches the solidus most closely. In any event, there is a clear implication that the viscous drag at the base of shield lithosphere is much higher than elsewhere—a prediction which receives some support from observation.

Finally, it is clear that the thickness of the lithosphere, both continental and oceanic, increases (not linearly) with age. This implies that shield lithosphere was once much thinner than it is now and that the asthenosphere beneath it was thicker, thus facilitating continental drift during the Precambrian. As for the future, continental lithosphere will presumably continue to thicken, increasing the viscous drag at its base and thus gradually bringing drift to a halt. □

Communal nesting in birds

from John Krebs

THE Central and South American Groove-billed Ani (*Crotophaga sulcirostris*) lives in groups of one to four monogamous pairs. All the females in a group lay their eggs in a communal nest, and both members of each pair help to incubate and feed the young. The axiom of selfish genery warns one to expect that Ani groups are uneasy alliances in which each individual pursues its own reproductive ends. A study recently reported by S. L. Vehrencamp (*Science* 197, 403; 1977) reveals that this is indeed the case. The most obvious and unequivocal sign of self interest is that the females in a group roll each other's eggs out of the communal nest. Females cannot, however, distinguish their own eggs from those of others so that an individual only engages in egg-rolling before she herself has started to lay. This means, of course, that the first female to start

laying eggs is more likely to suffer, and the last female to start loses no eggs. Although the first female(s) to lay to some extent compensate for egg loss by laying larger clutches, they still end up with fewer eggs than the last female at the time of incubation. In comparing nesting groups of different sizes, Vehrencamp found that the average number of eggs per female is positively correlated with group size, but the first female to lay in the group raises fewer young than a solitary pair, while the last female raises more. So far all the benefits seem to lie with the last female to lay and none with the first. There is potentially a cost to the late layer, because the first female starts to incubate as soon as she has completed her clutch, and the first young to hatch out survive better than later ones. The late female's answer to this is to lay her eggs more rapidly, at 1–2 day intervals compared with 2–3 days for early females, so that her young hatch out no later than average. All in all, the late females clearly do better than early layers, and they tend to be older (probably more dominant) individuals who are mated to larger than average males.

The late layers also gain when it comes to parental care: they contribute less to incubation and feeding the young than do the other females in a group even though most of the brood is theirs. This picture of more or less parasitic behaviour by late females is to some extent ameliorated by the fact that the males mated to late layers do more incubation and feeding of the young than any other group member. This implies that the male can tell that his mate has laid most of the successful eggs so that he is feeding his own children.

Clearly we need to know a lot more about the Ani system of communal nesting before a full cost benefit analysis for each group member can be done. At the moment it looks as though the late-laying female and her mate get more out of the system than the others, so further data are needed to work out why the communal nesting system is a stable, if selfish alliance.

In other communal nesting species which have been studied over several years, the members of a group are usually closely related to one another. However, these species, including babblers of the genus *Turdoides* and two American jays (*Aphelocoma* spp.), have a different system of communal nesting from that of the anis. Only one pair does all the reproducing, and the other group members are usually subadults, many of them offspring of the reproductive pair and hence siblings of the present nestlings. Although the helpers, as they are called, may in-

dators, and feed nestlings, there is some uncertainty about how much they actually increase the reproductive success of the breeding pair (Zahavi *Ibis* 116, 84; 1974; Woolfenden *Auk* 92, 1; 1975; Gaston, thesis, Oxford, Univ. 1976). One interpretation (Gaston *op. cit.*) is that the helpers are providing just enough aid to encourage the parents to allow them to stay in their territory. In both the babblers and jays, young birds have rather little chance of establishing their own territory away from home, because the suitable habitat is fully occupied by adults, who have a low mortality rate. The best bet for a young bird is to stay at home and try to take over the parental territory when one or both of the parents die.

In doing this, the young birds could reduce the future reproductive success of their parents by competing for food, attracting predators to the nest, and so on. In order to offset this, and hence make it profitable for the parents to allow them to stay, they provide a small amount of help in rearing new young.

This interpretation is speculative, but it emphasises that the apparently simple cooperation probably has a rich and fascinating underlying pattern of selfish behaviour □



A hundred years ago

METEOROLOGY AND THE INDIAN FAMINE . . . Dr Hunter also found that six great scarcities of sufficient gravity to be officially returned as "famines" had occurred during the period 1880–77. Of these six famines five were caused by years of drought coincident with, or adjoining to, the periods of minimum sunspots. He further showed that the rainfall at Madras passed through an eleven year's cycle, corresponding with the cycle of sun-spots. That is to say, the rainfall reaches its minimum in the eleventh year, rises to its maximum about half-way through the cycle in the fifth year, and then declines again to its minimum in the eleventh year.

At the recent biennial meeting of the German Astronomical Society, which was held at Stockholm, the members received the news of the discovery of the two satellites of Mars with manifestations of grave doubts. The president at their request telegraphed to the Berlin Observatory, and in reply received a copy of the original telegram as it was sent from America.

From *Nature* 16, 13 September, 425, 428; 1877.

John Krebs is at the Edward Grey Institute of Field Ornithology at the University of Oxford.

Carbon fixation pathways

from Sandy Grimwade

A symposium on the C_3 and C_4 photosynthetic pathways was held by the Ecological Society of America at the Annual Meeting of the American Institute of Biological Science in East Lansing, Michigan in August 1977.

THE fixation of atmospheric carbon dioxide into metabolic intermediates by green plants can take place by three different pathways. In the majority of plants CO_2 is fixed by the classic Calvin cycle directly into ribulose diphosphate to form two molecules of phosphoglycerate—a three-carbon compound. In some plants, however, the initial carboxylation step results in the formation of C_4 compounds such as oxaloacetate, which are then decarboxylated in specialised bundle sheath cells where the released CO_2 is refixed by the usual Calvin cycle. The third, and most restricted type is found mostly in succulents of arid regions. Fixation of CO_2 in this case occurs into crassulacean acid.

The differences between the so-called C_3 and C_4 plants give rise to a wide variety of biochemical, ecological and climatological implications, some of which were discussed at the symposium.

B. N. Smith (Brigham Young University) outlined the biology of C_3 and C_4 plants and the resulting isotope fractionation effects. Atmospheric CO_2 contains both the ^{12}C and ^{13}C stable isotopes of carbon. Compared with a standard limestone sample, the relative concentration of the ^{13}C isotope in atmospheric CO_2 ($\delta^{13}C$) is -7% . The photosynthetic fixation of atmospheric CO_2 exhibits a substantial isotope effect, which differs markedly for C_3 and C_4 plants. As CO_2 fixation is the only reaction which shows such a substantial effect, the resulting isotope depletions are reflected throughout the plant tissues. In C_3 and C_4 plants, the $\delta^{13}C$ values are -26 and -12% respectively. In addition, these isotope depletions are maintained in the herbivores which eat the plants and so on up the food chain. As these differences are so marked, they provide a valuable tool in several fields. For instance, the relative concentration of C_3 and C_4 plants in fossil animals can be estimated. The adulteration of honey—mostly from C_3 plants—with

corn syrup, from a C_4 plant, could be detected and quantified.

Any consideration of C_3 and C_4 metabolism in plants gives rise to two related questions—why do plants use more than one pathway for CO_2 fixation and how did the pathways evolve. Answers to these questions must account for the fact that C_4 metabolism is found spread throughout many plant genera, and yet no genus is composed exclusively of C_4 plants. For instance, about 50% of grass species are C_4 plants as is a smaller proportion of Compositae. The only way to account for such a patchy distribution is to hypothesise that C_4 metabolism with its attendant array of specialised enzymes and cellular anatomy arose as many as 20 times in the course of plant evolution. The alternative proposition, that it arose once or a few times is almost impossible to reconcile with accepted schemes.

The question of the rationale for more than one pathway is easier to tackle. J. R. Ehleringer (Stanford University) presented data showing the advantages of C_4 metabolism in grasses in particular environmental circumstances. Measurements on whole plants of net carbon gain per unit weight show the following. At normal oxygen tension, C_3 and C_4 plants show similar efficiencies, but as O_2 tension decreases, the efficiency of C_4 metabolism increases whereas C_3 remains constant. Similarly, the yield of C_4 plants is constant with increasing temperature whereas the efficiency of C_3 metabolism decreases. C_4 plants also have the advantage at high light intensities and low humidity. Using these data, a computer simulation of the rate of carbon gain in a C_3 or C_4 grass canopy in the Great Plains of the United States in July shows that as latitude increases, so does the efficiency of C_3 grasses. At $45^\circ N$ the two types are about equal. A survey of the flora of the Great Plains reveals that in the south of Texas ($25^\circ N$) about 70% of the grass cover is C_4 , at $40^\circ N$ the two types are equal, and at $60^\circ N$ only C_3 types are found. Thus the relative physiological advantage of C_3 or C_4 metabolism is directly reflected in the flora of the region.

Similarly, a survey by C. T. Harrison (University of Wyoming) of the grass types along a transect of grasslands in Wyoming shows a decrease in the percentage of the biomass composed of C_4 grasses with increasing elevation, and hence decreasing average temperature. This point is nicely reflected in measurements of the $\delta^{13}C$ values of bison remains in a single burial pit in Wyoming. At the surface, the $\delta^{13}C$ values reflect a diet for the bison which contains a substantial proportion of C_4 grasses. With increasing depth,

and hence age, the proportion of C_4 in the diet decreases until the $\delta^{13}C$ value reflects a diet composed entirely of C_3 plants approximately 8,000 years ago. This is held to reflect the gradual warming of the climate, with the subsequent invasion of C_4 grasses from the south over the past few millennia. $\square$

Gravitation but no levitation

by Malcolm MacCallum

The Eighth International Conference on General Relativity and Gravitation was held at the University of Waterloo, Ontario, Canada on 7–12 August, 1977. The organising committees, headed by M. McKiernan (University of Waterloo) and W. Israel (University of Alberta, Edmonton), provided a most enjoyable and enlightening meeting.

'GR8' proclaimed the T-shirts: they were right in more ways than one. Up to nine parallel afternoon sessions were required to accommodate more than 400 talks contributed from among the 700 or so participants. The morning plenary sessions were devoted to invited talks, mainly reviews.

Informal discussions between V. Braginsky (Moscow), W. Unruh (University of British Columbia, Vancouver) and others evolved schemes to push the sensitivity of gravity wave detectors to the level where the instrument must be regarded as a quantum system. A factor of about one million improvement is required to measure the expected astrophysical sources, as described by K. S. Thorne (Caltech). The active groups developing the various possibilities were all represented, and there seemed to be considerable optimism about the feasibility of the new detectors and the likely technological spin-off from their development.

Experimental results by I. S. Shapiro (Massachusetts Institute of Technology) on time-delay data from the Viking missions to Mars, and by R. B. Partridge (Haverford College) on the isotropy of the cosmic microwave background contained no real surprises, apart from some unexplained and unsystematic deviations in Shapiro's data which he tentatively ascribed to procedural errors at the tracking stations. R. Newman (University of California,

Irvine) described new laboratory tests confirming the inverse square law at ranges around 4 cm in contradiction to some results of Long (*Nature* **260**, 417; 1976).

The ever-expanding capabilities of computers were much in evidence, and I suspect many relativists will have been converted to a new interest in the potential of the algebraic manipulation systems, most of which, such as the SHEEP system demonstrated by I. Frick (University of Stockholm), are designed for ease of casual use. Interesting numerical computations included two splendid films, by J. R. Wilson (Lawrence Livermore Laboratory) on collapse and by L. Smarr (Harvard University) and K. Eppley (University of Maryland) on the head-on collision of two equal mass black holes. Thorne suggested that generalisation of the latter to glancing incidence might increase the rather low gravitational radiation found by Smarr and Eppley.

The significance of black holes for astrophysics and cosmology was well reviewed by M. J. Rees (Institute of Astronomy, Cambridge) and I. D. Novikov (Moscow) respectively. Effects considered ranged from X rays through quasar models to the overall entropy of the Universe, and included the polarisation effects recently predicted by P. A. Connors and R. F. Stark of the University of Oxford (*Nature* **266**, 429; 1977). Among the other astrophysical contributions, J. L. Friedman (Wisconsin-Milwaukee) announced that all rotating stars are unstable, and C. M. Caves (Caltech) pointed out that gravitational Cerenkov radiation would cut off the cosmic ray spectrum in some alternative theories of gravity.

The singularities symposium consisted of many informal contributions, shorter even than their written abstracts, carefully organised by G. F. R. Ellis (University of Capetown) into an enlightening overview. The difficulties in defining singular boundary points and in formulating the 'cosmic censorship' hypothesis (roughly, that all singularities other than an initial big bang are hidden inside black holes) were well discussed. In the session on the initial-value problem Y. Choquet-Bruhat (Paris) reviewed her work with J. Marsden (University of California, Berkeley) proving that the energy of asymptotically flat spaces has a minimum, namely zero, at flat space, thus partially verifying the long-standing conjecture that such spaces have positive energy.

Complex variable techniques figured in various contexts. W. Kinnersley and D. M. Chitre (Montana State University) have developed a method for generating infinite families of solutions

of Einstein's equations. R. Penrose (University of Oxford), in his review, related his twistor programme to the soliton solutions of Yang-Mills theory.

The various schools of thought on quantum gravity were well represented. The most rapidly growing seems to be 'supergravity', in which gravity is related to a spin-3/2 field through a symmetry. This removes some divergences, has positive energy, and, as shown by R. Tabensky and C. Teitelboim (Princeton University), can be regarded as a 'square-root' of general relativity much as Dirac's equation is related to the Klein-Gordon equation. S. W. Hawking (University of Cambridge), speaking on path-integral formulations, pictured the vacuum as a sea of Planck mass black holes and warned 'Danger! Virtual black holes.' The gap between quantum and classical approaches was still apparent, as when S. Weinberg (Massachusetts Institute of Technology) told a questioner that geometry did not matter, and only belatedly added 'in this case'.

One interesting contribution that did not materialise was by L. Domash (Maharishi University) on levitation. Did his theory break down in mid-Atlantic? Anyway, only lesser adepts in meditation were present. □

Oocyte microinjection

from H. Chantrenne

A symposium on the use of *Xenopus* oocytes in the study of DNA transcription and mRNA translation was held on 16 July, 1977 at the Université Libre de Bruxelles as part of an EMBO course. It was organised by Dr G. Marbaix and Dr G. Huez, Laboratoire de Chimie Biologique, Département de Biologie Moléculaire, Université Libre de Bruxelles.

THE colloquium presented some recent results obtained with *Xenopus* oocytes. J. Brachet (Brussels) provided an introduction to oocyte structure and oogenesis.

A novel development is the use of oocytes to study the expression of injected DNA. J. B. Gurdon (MRC Laboratory of Molecular Biology, Cambridge) described techniques he has developed for studying the expression of purified genes by injecting the DNA into the oocyte germinal vesicle, where

it is transcribed. He also described recent experiments showing that the oocyte cytoplasm can 'reprogram' injected nuclei. Nuclei from fully-differentiated *Xenopus* cells injected into oocytes of the newt *Pleurodeles*, directed the synthesis of proteins characteristic of the *Pleurodeles* oocyte stage rather than proteins characteristic of the differentiated cell (see *Proc. natn. Acad. Sci. U.S.A.* **74**, 2470; 1977).

The oocyte provides an alternative to the more usual cell-free systems for studying the translation of mRNA. L. van Vloten-Doting (State University, Leiden) emphasised the advantage of the oocyte. Translation is correct, the oocyte is of course able to adjust the conditions for adequate operation of the complex protein-synthesising machinery, and translation continues for several days. Another remarkable feature is the oocyte's ability to bring about correct post-transcriptional modification of the newly-synthesised polypeptide.

An illustration of this was given by J. Ghysdael (Brussels) with the 35S RNA from avian myeloblastosis virus. When injected into oocytes this RNA directs the synthesis of a long polypeptide which is the precursor of the 'group antigens' of the virus. In the oocytes it is correctly split into four viral proteins (p27, p19, p15, p12) exactly as in infected chick fibroblasts, but much more slowly, allowing a clear analysis of the process by classical chasing methods. According to C. von der Helm (see *Proc. natn. Acad. Sci. U.S.A.* **74**, 911; 1977) protein p15, one of the final products of polypeptide cleavage, is involved in the cleavage. This raises an intriguing problem, for p15 is not initially present in the oocyte; no-one knows at present whether it can act enzymatically when it is still part of the large polypeptide and provided that the concentration of the latter is sufficient, or whether selective cleavage is brought about by oocyte enzymes.

G. Huez and G. Marbaix (Brussels) have been studying the role of poly(A) in the stability of the messenger. They showed that the addition of poly(A) to a histone mRNA lacking poly(A) increases its half-life in the oocyte from around 10 to more than 48 hours. G. Vassart (Brussels) showed how the translation of thyroglobulin mRNA in oocytes has resolved the controversy over the size of the protein subunit, which turns out to have a molecular weight of 300,000. It is only in the oocyte that this messenger is translated correctly. B. Lebleu (Brussels) showed that crude preparations of mRNA from cells induced to produce interferon directed the synthesis of biologically active interferon in the oocyte. □

H. Chantrenne is Professor of Biochemistry in the Faculty of Sciences, Université Libre de Bruxelles.

articles

Ultraviolet spectrum of quasi-stellar object 3C273

Arthur F. Davidsen, George F. Hartig & William G. Fastie

Department of Physics, The Johns Hopkins University, Baltimore, Maryland 21218

The first direct observation of the ultraviolet spectrum of a quasi-stellar object (QSO) has been made with a rocket-borne telescope. The emission line spectrum of 3C273 is similar to the spectra of high-redshift QSOs, but no absorption is observed. The results provide important new constraints on theoretical models of QSOs, place a severe limit on the density of neutral hydrogen in the intergalactic medium, and suggest a cosmological origin for much of the absorption seen in high-redshift QSOs. Comparison of the ultraviolet spectrophotometry of low- and high-redshift QSOs suggests that the universe is closed, with $q_0 \sim 1$.

It has long been recognised that ultraviolet observations of low-redshift quasi-stellar objects (QSOs) made with space-borne telescopes might reveal important clues concerning the nature of these objects. Among the problems for which such observations are relevant are the nature of the energy source, the physical conditions in the emission line envelopes, and the origin of the absorption lines seen in high-redshift QSOs. It now seems that these observations may also be of great significance for cosmology. Here we report the first observation of the ultraviolet spectrum of a QSO, made with a telescope carried above the Earth's atmosphere by a sounding rocket. The spectrum of 3C273 is presented and some of its implications are briefly discussed. Further details of the observation and analysis will be described elsewhere¹.

Faint object telescope

The observation was made with a new instrument², which we call the Faint Object Telescope (FOT), designed especially for moderate resolution (10–15 Å) ultraviolet spectrophotometry of faint extragalactic objects in the wavelength region between 1,200 and 1,700 Å. The FOT uses a 40-cm diameter $f/15$ telescope, whose 1,100 cm² collecting area makes it the largest optical telescope ever flown in a sounding rocket. A spectrum is formed by a concave grating which gives a dispersion of 20 Å mm⁻¹ at the detector focal plane. The spectrograph has two circular entrance apertures whose diameters, projected on the sky, are 2.2 arc min with a centre-to-centre spacing of 4.4 arc min. One aperture observes the target while the other accumulates simultaneous background data.

The FOT detector consists of two 25-mm diameter micro-channel plates mounted in a chevron configuration, followed by two one-dimensional resistive anode readout devices^{3–5} for accumulating separate spectra of the target and background. The detector has a magnesium fluoride window, and a caesium iodide photocathode is deposited directly on the front surface of the first microchannel plate. Pressure in the detector is maintained below 10⁻⁷ torr by an ion pump which is turned

off before flight. The spectrum observed in the target channel is displayed on the ground in real time by a multichannel analyser.

The overall effective collecting area of the FOT varies between 11 and 5 cm² over the range 1,250–1,700 Å. Pre-flight and post-flight calibrations agree within the errors of less than 5%. The absolute calibration, obtained by comparison with measurements made with an NBS-calibrated photodiode, is accurate to about 10%. The instrumental resolution is about 10 Å over most of the spectrum and degrades to about 15 Å at the shortest wavelengths.

A unique rocket pointing system has been developed for the FOT by the Sounding Rocket Division of NASA's Goddard Space Flight Center. Two star-trackers are mounted behind the telescope primary, with adjustable mirrors which enable them to view out through the side of the rocket. These star-trackers lock on to two bright stars which are chosen to be nearly orthogonal to the target direction and to each other. Signals from one tracker control yaw and roll jets in the attitude control system, while the second tracker controls pitch. With accurate alignment of the trackers and telescope, pointing within 1 arc min to any target can be accomplished.

In addition to this system, the FOT utilises an SIT vidicon television camera which is focused on the mirrored entrance plate of the spectrograph. Television pictures of a 15-arc min field surrounding the target and background apertures are transmitted to the ground, where the experimenter can verify the pointing of the telescope by reference to stars as faint as 10 mag. A remote command system can then be used to make fine adjustments to the pointing. The operation of the FOT is, therefore, somewhat similar to the operation of large ground-based telescopes.

Launch and data

The Faint Object Telescope was launched aboard a NASA Black Brant VC sounding rocket from White Sands Missile Range on 16 April 1977 at 0500 UT. The star-trackers acquired Vega and Capella and pointed the FOT so that 3C273 was within 50 arc s of the centre of the target aperture. A series of commands were then sent to centre the target in the spectrograph aperture. Pointing was automatically maintained throughout the rest of the flight with a total jitter of about ± 15 arc s. The payload reached a peak altitude of 218 km and obtained a total of 270 s of data at altitudes above 130 km.

Some 13,000 photons were detected from 3C273 during a 235-s observation after the target was centred in the spectrograph entrance aperture. The total background correction was $\sim 2,000$ counts, determined from simultaneous observations through the sky aperture as well as observations made with the target aperture before and after the 3C273 observation. About half of the background was due to dark counts distributed nearly uniformly over the whole spectrum. The other half was due to geocoronal Lyman α emission, for which the FOT has

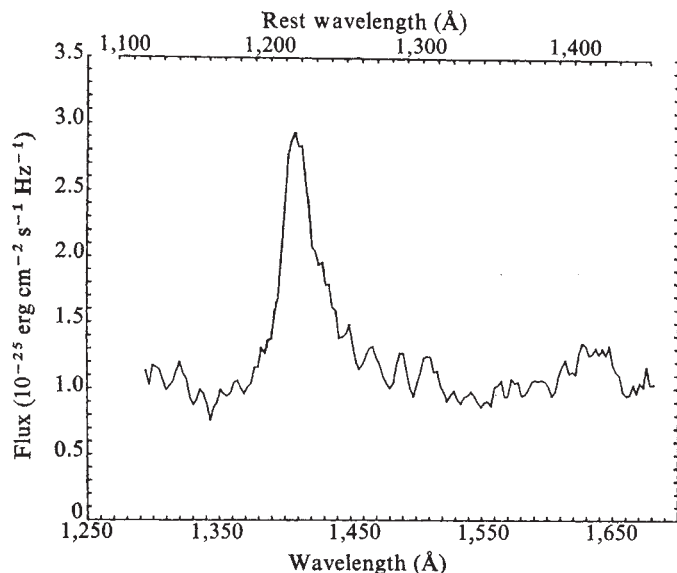


Fig. 1 The ultraviolet spectrum of the QSO 3C273. The strongest emission line is hydrogen Lyman α λ 1,216 with a redshift $z = 0.16$. The asymmetry of the line is attributed to N(v) λ 1,240 emission. The broad feature at λ_{rest} 1,400 Å is similar to that usually attributed to Si (iv) and O (iv) in high-redshift QSOs. There are also probable emission features which may be due to O (i) λ 1,304 and Si (ii) λ 1,265. The continuum is flat, with no evidence of strong absorption shortward of Lyman α .

a small residual sensitivity at detector positions corresponding to wavelengths shorter than 1,300 Å. The background corrected spectrum was smoothed with a sliding average of 3 wavelength bins (7.7 Å) to reduce the effects of slightly uneven bin widths in the analog-to-digital converter. The count-rate data were then reduced to absolute flux units by dividing by the response function of the FOT.

Emission line spectrum

The ultraviolet spectrum of 3C273 is shown in Fig. 1. As in the case of very high-redshift QSOs, the strongest emission feature is Lyman α . Its redshift, $z = 0.16$, is the same as the value obtained originally from the Balmer lines⁶. In addition there are emission features due to N(v) λ 1,240 (blended with La), Si(iv) and O(iv) λ 1,400, and O(i) λ 1,304. There are also possible emission features at λ 1,265 and λ 1,286. The first of these can plausibly be identified with Si(ii). The equivalent width of the La + N(v) feature is 67 Å in the rest frame. We estimate from the asymmetry of the feature that one quarter of this is due to N(v). The continuum is flat, with a flux of $9.5 \pm 1.0 \times 10^{-26}$ erg cm⁻² s⁻¹ Hz⁻¹. The flux in La is, therefore, about 9×10^{-12} erg cm⁻² s⁻¹. Better estimates of the emission line intensities and their widths will be available when we have had the opportunity to compare the data with the results of synthetic FOT spectra (see ref. 1).

Several attempts to reproduce QSO emission line spectra have been made, using a model consisting of a power-law source of ionising radiation surrounded by clouds or filaments of gas⁷⁻¹⁰. Until now, however, there have been no observations of a single QSO covering both the ultraviolet and the optical emission lines. Theorists have had to resort to a 'composite QSO spectrum' pieced together from observations of different rest-wavelengths in many QSOs of varying redshifts. The usual assumption for normalising the ultraviolet and optical data has been to take the flux ratio $F(\text{La})/F(\text{H}\beta) = 40$, corresponding to production of these lines primarily by radiative recombination, but with some enhancement of La due to collisional excitation. Combining our measurement of the La flux with the previously measured H β flux¹¹, we find $F(\text{La})/F(\text{H}\beta) \simeq 4$ in 3C273, an order of magnitude smaller than the canonical value. A similar result has been obtained in a very careful composite QSO study¹².

This remarkable departure of the observed Lyman/Balmer ratio from the expected one is likely to have a major impact on QSO models. The most obvious explanation, that the ultraviolet lines are attenuated by absorption by dust similar to that observed in the interstellar medium, seems untenable in view of observations of Paschen α that indicate the hydrogen lines are unreddened¹³. But, dust which is distributed within the line-emitting gas might destroy La without having much effect on the Balmer and Paschen lines if the nebula has high optical depth to La photons. An additional mechanism which might play a part in depleting La in an optically thick nebula is photoionisation of He 2³S by trapped La photons¹⁴. Some alternatives have been mentioned by Baldwin¹². Whatever mechanism is at work, an understanding of the reduced La/H β ratio may lead to vastly improved knowledge of the physical conditions in QSO envelopes.

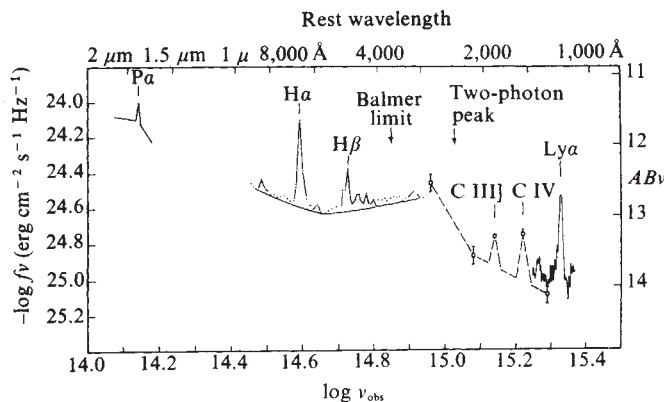
Non-thermal continuum

It is usually assumed that the optical continuum observed in QSOs and active galactic nuclei can be extrapolated to the far-ultraviolet with a power-law $F_{\nu} \propto \nu^{-\alpha}$ to provide the photoionising flux needed to explain the emission lines. Theoretical models of this type are sensitive to the flux at the Lyman limit as well as the spectral index α . This observation of 3C273 makes it the first QSO for which the continuum flux is well-determined over a broad wavelength range.

In Fig. 2 we have collected spectral data on 3C273 extending from the infrared to the far-ultraviolet. The data at 2 μ m are from Grasdalen¹⁸, the optical data are from Oke and Shields¹⁵, the near ultraviolet data are recent broad band measurements by the ANS satellite¹⁶, and the far-ultraviolet data are the measurements reported here. Two of the ANS bands fall at the expected wavelengths of the C(iv) λ 1,550 and C(III) λ 1,909 lines, which are normally strong in high-redshift QSOs.

From Pa to Ha the spectral index is $\alpha \simeq 1.0$, but between Ha and La the spectrum is flatter, with $\alpha = 0.6$. In addition there is a broad hump centred at $\sim 3,500$ Å. Such a feature has been noted in several QSOs by Baldwin¹¹, who has interpreted it as Balmer continuum emission. It is evident that the spectrum of 3C273 cannot be described by a single power-law, but because only a very small extrapolation is now required, the flux at the Lyman limit is reasonably well-determined. We estimate F_{ν} (Lyman limit) = 8×10^{-26} erg cm⁻² s⁻¹ Hz⁻¹, with an uncertainty of about 20%. This corresponds to a continuum luminosity $L_{\nu} = 7 \times 10^{30}$ erg cm⁻² s⁻¹ Hz⁻¹, assuming $H_0 = 50$ km s⁻¹ Mpc⁻¹ and $q_0 = +1$. The spectral index just below the Lyman limit is $0 \lesssim \alpha \lesssim 0.6$, but α must increase ($\alpha \gtrsim 1.2$) somewhere above the limit to agree with the X-ray observations¹⁷.

Fig. 2 The spectrum of 3C273 from the near-infrared to the far-ultraviolet. Infrared data¹⁸ are represented by a curve. Optical data are shown as measured fluxes and assumed continuum¹⁵. Five broad-band ultraviolet measurements by the ANS satellite¹⁶ are shown as squares, with dashed lines to indicate assumed continuum and lines. The far-ultraviolet data from Fig. 1 are also shown.



No corrections for reddening have been made to any of the data shown in Fig. 2. The chief evidence against reddening of the overall spectrum is the Pa/H β measurement¹³, which agrees with recombination theory. Galactic reddening is expected to be small or zero¹⁸ at the high galactic latitude ($b^{\text{II}} = 64^\circ$) of 3C273. An estimate based on the 21-cm hydrogen column density¹⁹ and the relation between N_{H} and reddening^{20,21} gives $E_{\text{B-V}}$ (galactic) = 0.01, which would give extinction of 0.1 mag at Lyman α using the OAO extinction curve²².

Intergalactic matter

The absence of absorption shortward of the red-shifted La emission is another important result of this observation. Gunn and Peterson²³ showed that neutral hydrogen distributed smoothly in the intergalactic medium should produce an absorption trough extending from 1,216 Å to 1,216 (1+z) Å in the spectrum of a QSO, assuming its redshift is cosmological. The optical depth in the trough at a wavelength corresponding to redshift z is²⁴

$$\tau = 1.7 \times 10^{11} \frac{n_{\text{H}}(z)}{(1+z)^{3/2}}$$

in a Friedmann model universe with the critical density parameter $\Omega = 1$. Since no trough is observed in the 3C273 spectrum, we take $\tau < 0.2$ and find the upper limit

$$n_{\text{H}} < 1.5 \times 10^{-12} \text{ cm}^{-3}$$

for $z \sim 0.07\text{--}0.15$. The contribution to the critical density due to neutral hydrogen is, therefore, $\Omega_{\text{HI}} < 5 \times 10^{-7}$ at $z \simeq 0.1$. This is by far the strongest limit on neutral hydrogen in the intergalactic medium at a recent epoch, but a similar limit has previously been obtained at $z \simeq 2$ from observations of high-redshift QSOs (see ref. 25 for a review). These results indicate that if there is any intergalactic gas it has remained highly ionised over most of the history of the Universe.

QSO absorption lines

Absorption lines have been seen in the spectra of many high-redshift QSOs and most of these are found shortward of the La emission line²⁵. It is highly controversial whether all of these lines arise in material associated with the QSOs, or whether some of the absorption is produced in intervening clouds or galaxies. On the intervening matter hypothesis, the absorption should be absent in low-redshift QSOs²⁶.

Absorption lines are not readily detectable at the modest resolution of the present observations of 3C273. It is still possible to make an interesting comparison between 3C273 and high-redshift QSOs, however, because even at low resolution, these objects usually show a marked depression of the apparent continuum shortward of La due to the blending of many absorption lines. For example 10 QSOs with $2.5 \leq z \leq 3.1$ have been well observed both longward and shortward of La by Osmer and Smith²⁷. For these objects we have estimated the ratio of the flux density in the wavelength range 1,120–1,180 Å to that at 1,475 Å (tabulated by Osmer and Smith). A wavelength region slightly longer than La was not chosen because of possible contamination by emission lines. The mean value of this ratio is 0.66, with a range from 0.51 to 0.81. Our observation of 3C273 yields 0.98 ± 0.06 for this ratio, and suggests that absorption lines will not be detected when high resolution observations of this object are eventually made. These results are, therefore, consistent with the hypothesis that much of the absorption seen in high-redshift QSOs is due to matter at smaller cosmological redshifts.

One difficulty with this comparison is that the high redshift objects are more luminous than 3C273 by a factor between 3 and 10, and there could conceivably be more absorption in higher luminosity objects if, for example, material is expelled by radiation pressure. A scan of OH 471 (Baldwin, J. A., personal communication), which has $z = 3.4$ and a continuum luminosity very similar to that of 3C273 for $q_0 = +1$, has,

Table 1 QSOs of similar luminosity

Object	z	AB_{v} (1,450 Å)	$\log W_{\lambda}$ (La+N(v))	$\log L_{\text{v}}$ (1,450 Å)
4C25.05	2.34	18.7 ± 0.2	1.84	30.93 ± 0.08
2256+017	2.66	19.3 ± 0.4	1.79	30.76 ± 0.16
4C05.34	2.86	18.3 ± 0.2	1.79	31.20 ± 0.08
0830+115	2.97	19.2 ± 0.2	1.87	30.86 ± 0.08
OH 471	3.39	19.1 ± 0.2	1.86	30.97 ± 0.08
Average	2.84	18.92 ± 0.37	1.83 ± 0.03	30.94 ± 0.15
3C273	0.158	13.96 ± 0.10	1.83 ± 0.04	30.94 ± 0.04

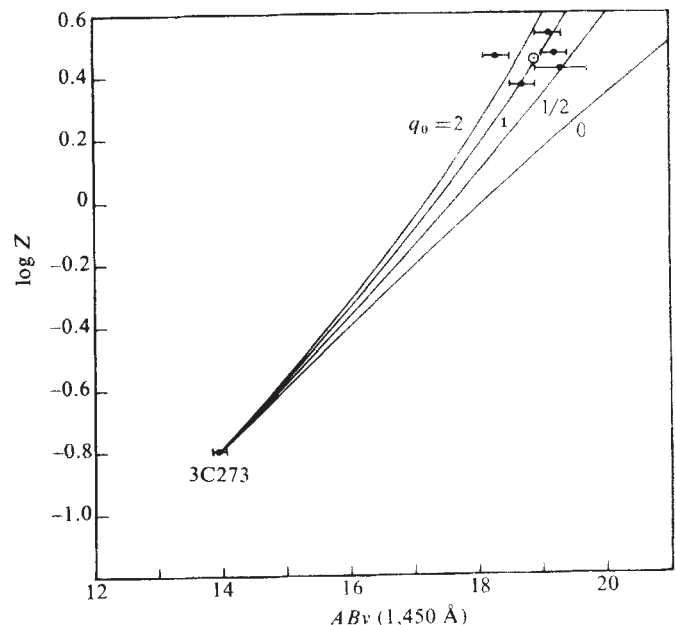
however, a continuum depression ratio of about 0.5, similar to that of the other high-redshift QSOs. Therefore, we tentatively conclude that the continuum depression ratio is correlated with redshift and that much of the absorption seen in high-redshift QSOs arises in matter at smaller, cosmological redshifts. It will be necessary to obtain further ultraviolet observations of intermediate-redshift QSOs, and eventually high resolution ultraviolet spectra, in order to confirm this conclusion.

Cosmology with QSOs

Quasars have been disappointing as cosmological probes, despite their high visibility at large redshifts, because if their redshifts are cosmological, they have a very large range of luminosities. It has recently been shown, however, that there is a good correlation between the continuum luminosity at a rest-wavelength of 1,450 Å, calculated under the assumption of cosmological redshifts, and the equivalent widths of the La+N(v) and C(IV) emission features in 20 high-redshift QSOs²⁸. Another study found a similar correlation with La (but not C(IV)) in 29 mostly radio-quiet QSOs²⁹. The relations are especially tight if the sample is restricted to only the flat spectrum radio sources²⁸, suggesting that these objects may be useful as standard candles.

In Table 1 we list the flat-spectrum radio QSOs from the study of Baldwin²⁸, which have equivalent widths W_{λ} (La+N(v)) within 10% of the value observed in 3C273. The continuum luminosities $L_{\text{v}}(1,450 \text{ Å})$ have been calculated under the assumption of cosmological redshifts with the Hubble constant $H_0 = 50 \text{ km s}^{-1} \text{ Mpc}^{-1}$ and the deceleration parameter $q_0 + 1$. Also listed are the apparent magnitudes $AB_{\text{v}}(1,450 \text{ Å})$ on

Fig. 3. A redshift-magnitude diagram for the QSOs in Table 1. Only flat-spectrum radio sources with W_{λ} (La+N(v)) similar to that of 3C273 are included. The circled point is the average of the five high-redshift objects. The curves show expected relationships for various values of the deceleration parameter q_0 in Friedmann model universes.



the Oke system ($AB_V = -2.5 \log F_V - 48.60$). The average of the five high-redshift QSOs has the same equivalent width as 3C273 and the same luminosity if $q_0 = +1$. These objects can, therefore, be compared directly in a redshift-magnitude diagram. The result is shown in Fig. 3.

Also shown in Fig. 3 are the expected redshift-magnitude relationships for various values of q_0 in Friedmann model universes with zero cosmological constant³⁰. These curves are arbitrarily normalised to go through the point representing 3C273. This comparison of high- and low-redshift QSOs thought to have similar intrinsic luminosities favours a value of $q_0 \approx +1$. If q_0 were greater than 0.5 it would indicate that the universe is closed.

There are two important cautionary notes in connection with this result. First, the reality of the equivalent widths of the ultraviolet emission lines as a luminosity indicator is not yet firmly established. This is because one also obtains a good correlation between apparent luminosity and equivalent width³⁸. This remains true with the addition of 3C273 to the sample if one considers observed equivalent widths rather than rest-frame values. Ultraviolet observations of several low-redshift QSOs and Seyfert galaxies with a wide range of luminosities are needed to decide whether the luminosity-equivalent width relationship is real.

A second uncertainty in the present result for q_0 is the reddening of 3C273. In Fig. 3, 3C273 is plotted with no correction for reddening of the Galaxy. If $E_{B-V} = 0.01$ as estimated above, the correction at 1,450 Å is less than 0.1 mag, which is small compared with other uncertainties. If, however $E_{B-V} = 0.06$, the ultra-violet magnitude should be brighter by about 0.5, which would give $q_0 \approx 0.5$. A larger reddening correction would reverse the evidence favouring a closed universe.

While it would be premature to conclude that the universe is closed, it is encouraging to think that QSOs may turn out to be useful cosmological probes. Further ultraviolet observations

may be expected to contribute to the solution of this problem, as well as to our understanding of QSOs and active galactic nuclei.

This work was supported by NASA under grant NGR 21-001-001. A.F.D. acknowledges the support of the Alfred P. Sloan Foundation. We thank H. Weiser for help in the design and construction of the detector. The electronics were built by Spacom Electronics, Camarillo, California. EMR, Princeton, N. J. applied the photocathode to the microchannel plate. The rocket was prepared and launched by the Sounding Rocket Division of the Goddard Space Flight Center and the U.S. Navy team at the White Sands Missile Range.

Received 12 August; accepted 19 August 1977.

- ¹ Davidson, A. F., Hartig, G. F. & Fastie, W. G. *Astrophys. J.* (submitted).
- ² Davidson, A. F., Hartig, G. F. & Fastie, W. G. *COSPAR/IAU Symp. New Instrumentation for Space Astronomy*, June, 1977, (in the press).
- ³ Lampton, M. & Paresce, F. *Rev. scient. Instrum.* **45**, 1098 (1974).
- ⁴ Lawrence, G. M. & Stone, E. J. *Rev. scient. Instrum.* **46**, 432 (1975).
- ⁵ Weiser, H., Vitz, R. C., Moos, H. W. & Weinstein, A. *Appl. Optics*, **15**, 3123 (1976).
- ⁶ Schmidt, M. *Nature* **197**, 1040 (1963).
- ⁷ Bahcall, J. N. & Kozlovsky, B.-Z. *Astrophys. J.* **155**, 1077 (1969).
- ⁸ Davidson, K. *Astrophys. J.* **171**, 213 (1972).
- ⁹ MacAlpine, G. M. *Astrophys. J.* **175**, 11 (1972).
- ¹⁰ Chan, Y.-W. T. & Burbidge, E. M. *Astrophys. J.* **198**, 45 (1975).
- ¹¹ Baldwin, J. A. *Astrophys. J.* **201**, 26 (1975).
- ¹² Baldwin, J. A. *Mon. Not. R. astr. Soc.* **178**, 67P (1977).
- ¹³ Grasdaen, G. L. *Astrophys. J.* **208**, L11 (1976).
- ¹⁴ MacAlpine, G. M. *Astrophys. J.* **204**, 694 (1976).
- ¹⁵ Oke, J. B. & Shields, G. A. *Astrophys. J.* **207**, 713 (1976).
- ¹⁶ Wu, C. C. *Bull. Am. astr. Soc.* **9**, 296 (1977).
- ¹⁷ Sanford, P. W. paper presented at the 8th Texas Symp. Relativistic Astrophysics (1977).
- ¹⁸ Sandage, A. *Astrophys. J.* **183**, 711 (1973).
- ¹⁹ Williams, D. R. W. in *Quasi-stellar Sources and Gravitational Collapse*, 213 (University of Chicago Press, Chicago, 1965).
- ²⁰ Gorenstein, P. *Astrophys. J.* **198**, 95 (1975).
- ²¹ Ryter, C., Cesarsky, C. J. & Adouze, J. *Astrophys. J.* **198**, 103 (1975).
- ²² Code, A. D., Davis, J., Bless, R. C. & Hanbury Brown, R. *Astrophys. J.* **203**, 417 (1976).
- ²³ Gunn, J. E. & Peterson, B. A. *Astrophys. J.* **142**, 1633 (1965).
- ²⁴ Field, G. B. A. *Rev. Astr. Astrophys.* **10**, 227 (1972).
- ²⁵ Strittmatter, P. A. & Williams, R. E. A. *Rev. Astr. Astrophys.* **14**, 307 (1976).
- ²⁶ Bahcall, J. N. *Astr. J.* **76**, 283 (1971).
- ²⁷ Osmer, P. S. & Smith, M. G. *Astrophys. J.* **210**, 267 (1976).
- ²⁸ Baldwin, J. A. *Astrophys. J.* **214**, 679 (1977).
- ²⁹ Osmer, P. S. *Astrophys. J.* **214**, 1 (1977).
- ³⁰ Sandage, A. *Astrophys. J.* **133**, 255 (1961).

Future sea-level changes due to West Antarctic ice sheet fluctuations

J. A. Clark

Cooperative Institute for Research in Environmental Sciences and Institute of Arctic and Alpine Research, University of Colorado, Boulder, Colorado 80309

C. S. Lingle

Department of Geological Sciences, University of Maine at Orono, Orono, Maine 04473

Global sea-level changes which would result from an instantaneous uniform thinning of the possibly unstable West Antarctic ice sheet are calculated and found to be non-uniform. At locations distant from the ice sheet (Hawaii, New York, the North Sea), immediate submergence would be followed by gradual emergence. At New Zealand, immediate submergence would be followed by gradual additional submergence, then slow emergence would begin after 2,500 yr. At locations close to the ice sheet (Cape Horn, the Ross Ice Shelf), the sea level would fall for 1,100 yr. then rapid submergence would start resulting in a net sea-level rise after 10,000 yr equal to about 92% of the average global rise.

It is commonly believed that the rise in sea level due to a glacial melting episode is uniform everywhere. That is, the observed sea-level rise, S , due to the melting of a mass of ice M_i is $S = M_i/\rho_w A$ with ρ_w the density of water and A the area of the oceans. This is

wrong because the ocean floor deforms under the weight of the changing ice and water loads and the ocean surface (the geoid) distorts as mass is redistributed on the surface, as well as within, the Earth. The observed change in sea level is the change in separation between the geoid and the ocean floor and Farrell and Clark¹ show that, when glaciers melt, the change in separation between these dynamic surfaces is not constant. We use their method in this analysis to predict changes in sea level which would result from melting (or accretion) of a uniform amount of ice from the relatively unstable West Antarctic ice sheet.

Instability in the West Antarctic ice sheet

The West Antarctic ice sheet (WIS), that portion of the Antarctic ice sheet lying in the Western Hemisphere and mostly east of the Transantarctic Mountains, was shown to be grounded extensively below sea level by seismic studies completed before 1961 (ref. 2). Weertman³ has shown that such marine ice sheets are not inherently stable, and Hughes⁴ has discussed several lines of evidence which indicate that WIS has disintegrated extensively

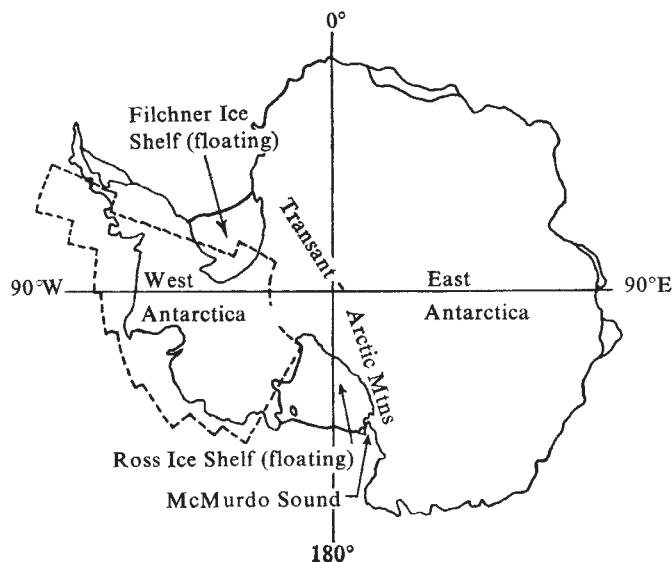


Fig. 1 The marine portion of the West Antarctic ice sheet (WIS) lies in the Western Hemisphere east of the Transantarctic Mountains. It is approximated for purposes of the numerical calculation by the area within the dashed line. The global sea-level changes which would result from a uniform thinning of the ice within this area are calculated.

since 10,000 yr BP, and has suggested that the process may be continuing.

Denton and Borns⁵ found that grounding of WIS in the vicinity of McMurdo Sound occurred before 47,000 yr BP. Following expansion, WIS remained grounded to the edge of the continental shelf until about 18,000 BP (ref. 5) (G. H. Denton, private communication) and then began retreating. WIS ungrounded once again in the McMurdo area by 6,000 yr BP (ref. 6). Thomas⁷ also suggests that accumulation rates near the West Antarctic ice divide imply continuous thickening of the WIS interior until 2,000 yr BP when thinning started, possibly as a delayed response to retreat of the grounding line.

Recent survey measurements made by Thomas⁷ on the Ross Ice Shelf indicate that part of the WIS grounding line may be advancing. If this is the case, the disintegration may be reversing, and WIS may now be expanding. The apparent inability of WIS to attain a state of equilibrium accords with the theory³ that predicts a marine ice sheet will be stable only after advancing to the edge of a continental shelf, or until water at the margin reaches a critical depth.

The model

The Earth is assumed to be a spherically symmetric, layered, self-gravitating, Maxwell viscoelastic solid. The elastic structure is that of a Gutenberg–Bullen Earth model with a uniform mantle viscosity of 10^{22} P and an inviscid core. Such a model has been suggested by others^{9,10} to closely approximate the real Earth.

The procedure, thoroughly described by Farrell and Clark¹ uses a Green function, $G(r-r_0, t-\tau)$, calculated by Peltier⁸ for the above Earth model. This Green function gives the effect of a 1-kg point load placed on the Earth's surface at position r and time τ upon relative sea level s at some other position r_0 and some later time t . To determine the relative sea-level change resulting from a distributed load that varies through time, consider the load to be a collection of point loads and add the effects of each. The relative sea-level change is then

$$s(r_0, t) = \int_{-\infty}^t \left(\iint_{\text{Load}} G(r-r_0, t-\tau) L(r, \tau) dr \right) d\tau \quad (1)$$

with L the surface load function. The load is comprised of a water load (L_w), and an ice load (L_i) so that

$$L = L_w + L_i = \rho_w s + \rho_i I \quad (2)$$

with ρ_w the density of water, ρ_i the density of ice, and I the ice thickness. Furthermore, the Green function can be separated into a time-independent elastic part (G_E) and a time-dependent viscous part (G_V)

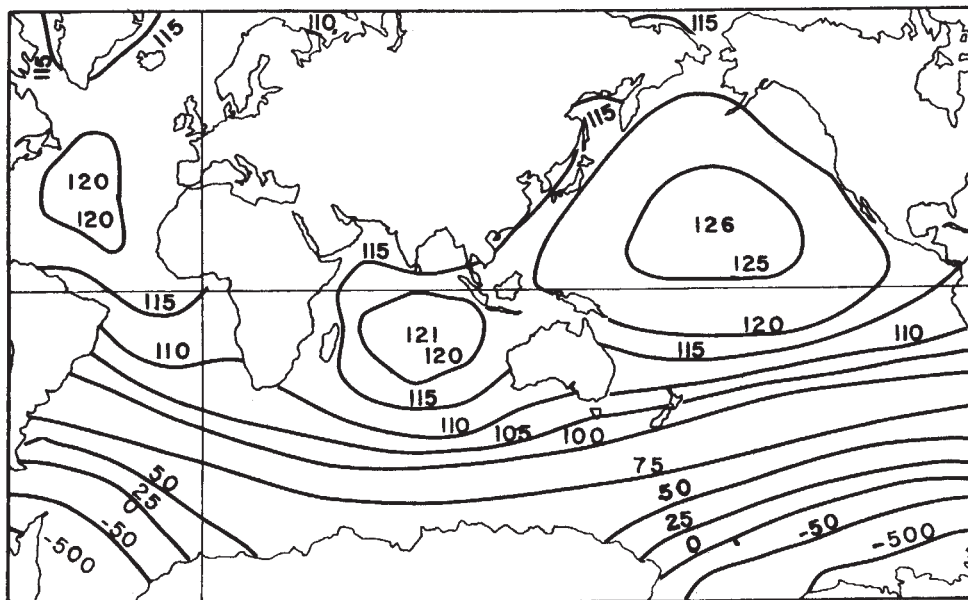
$$G = G_E + G_V \quad (3)$$

Substituting equations (2) and (3) into equation (1) gives

$$s(r_0, t) = \iint G_E(r-r_0) \rho_w s(r, t) dr + \iint_{\text{Ice}} G_E(r-r_0) \rho_i I(r, t) dr + \int_{-\infty}^t \left(\iint_{\text{Load}} G_V(r-r_0, t-\tau) L(r, \tau) d\tau \right) \quad (4)$$

The last term in equation (4) describes the time dependent viscous response of the Earth (the 'isostatic' response) to past ice and water load changes and the second term accounts for the immediate elastic response of the Earth to changing ice loads. The first term in equation (4) describes the Earth's elastic response to water load changes. Because s appears on both sides of equation (4), it is an integral equation describing a feedback process where meltwater

Fig. 2 Sea level changes everywhere immediately after a uniform thinning of WIS are shown as per cent of average global sea level rise (volume of water added to ocean-ocean area). The non-uniform distribution of sea level change is caused by the Earth's immediate elastic response to changed ice and water loading, plus the change in gravitational attraction exerted by WIS on the surrounding ocean. (The 100% contour corresponds to the 0.69 cm average oceanwide rise which would result from uniform thinning of WIS by 1 m.



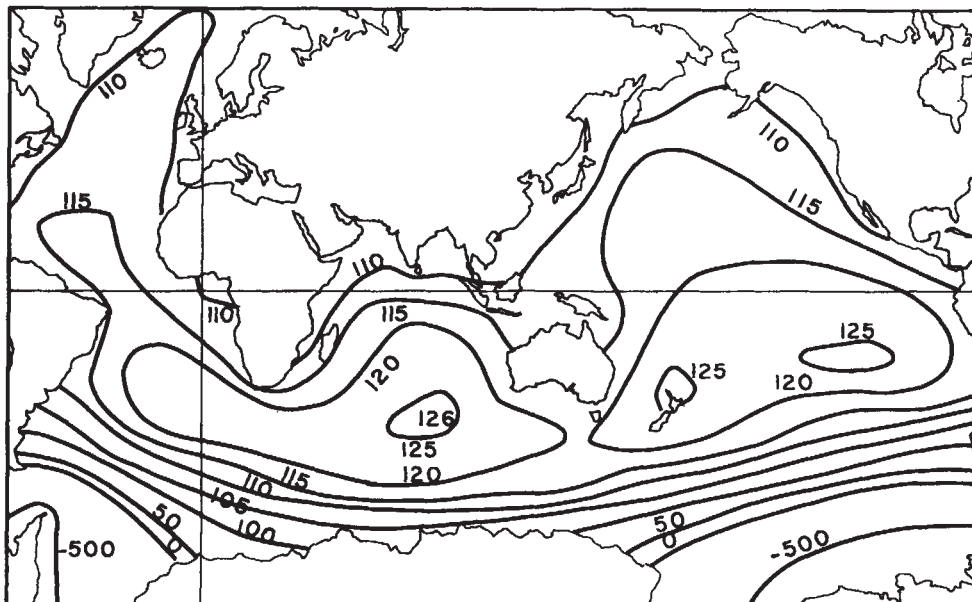
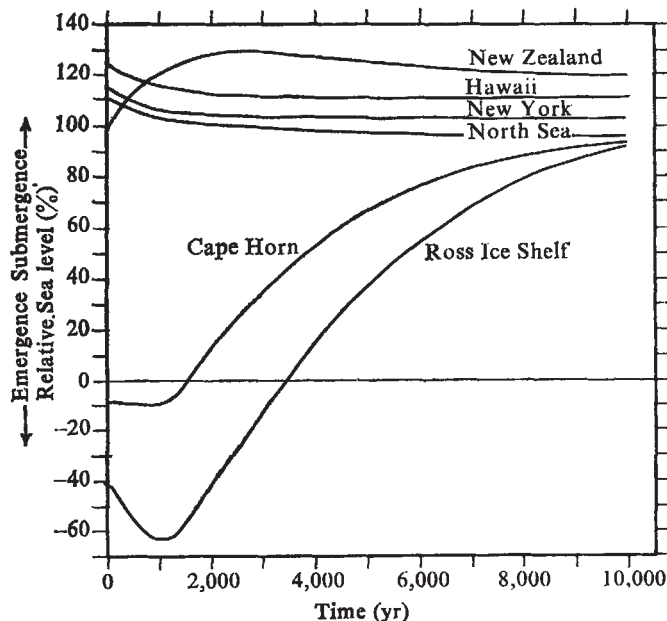


Fig. 3 Sea level changes everywhere 1,000 yr after the assumed uniform thinning of WIS, again shown as a percentage of average oceanwide rise. The new distribution of values results from gradual flow of material within the Earth's mantle

deforms the Earth and then flows to fill the resulting depressions in the ocean surface. This changes the water load and the process is repeated until equilibrium is attained. Equation (4) can be solved numerically, with realistic ice and ocean configurations, for the relative sea level change at any location and at any time.

The ice thinning model is a simple one in which 1 m of ice is uniformly removed from the entire WIS (see Fig. 1). The oceanwide average sea level rise, S , is then 0.69 cm. The rheological model is linear and so a doubling, for instance, of our assumed amount of uniform thinning results in a doubling of the predicted sea level changes everywhere. Furthermore, if WIS should thicken by 1 m instead of thinning, the predicted sea level changes would be identical in magnitude but opposite in sign to those given below. Our results are, therefore, quite general.

Fig. 4 Sea level change against time at six selected locations, for 10,000 yr following the assumed uniform thinning of WIS. At New Zealand, immediate submergence nearly equal to the average global rise is followed by continued submergence for 2,500 yr. Slight emergence follows. 10,000 yr later, net submergence is 120% of average global rise. At Hawaii, New York and the North Sea, immediate submergence of 125% to 110% is followed by emergence of 15% to 13%. At Cape Horn and near the Ross Ice Shelf, the sea level actually falls by 8% and 40%, respectively. Emergence continues for about 1,200 yr, after which sea level begins to rise. After 10,000 yr, net submergence is about 92% of average global rise.



Results

The results are given as the changes in sea level which would be caused solely by thinning of WIS. Tectonic effects and effects due to changes in glaciers elsewhere are not included. All reported values are in per cent of S , the oceanwide average sea level change.

Figure 2 shows changes in sea level everywhere immediately after the ice has melted. On Hawaii, for example, sea level rises 125% of S ($1.25 \times 0.69 = 0.86$ cm for the 1 m thinning case), while near the Ross Ice Shelf, the negative values indicate that sea level actually falls by 40%. This fall in sea level occurs despite the fact that water was added to the ocean; it is caused by the combined effects of immediate elastic uplift of the ocean floor near the ice sheet¹¹ and reduced gravitational attraction of the ocean water by the ice mass^{12,13}. Only along the 100% contour does the observed sea level rise equal the oceanwide average rise S .

After the Earth's immediate elastic response, viscous flow of material within the mantle slowly compensates for the new distribution of ice and water loads. Figure 3 shows the distribution of sea level changes (relative to present sea level) after 1,000 yr have elapsed. The sea level change at Hawaii is now only 116%, indicating that after the initial dramatic rise of 125% sea level slowly falls by 9%, resulting in slight emergence. In the extreme South Atlantic, however, where sea level rises only 50% initially (Fig. 2), Earth relaxation results in continual submergence so that after 1,000 yr sea level has risen to 100% of S . Figure 4 shows sea level change against time at six localities (New Zealand, Hawaii, New York, the North Sea, Cape Horn, and the Ross Ice Shelf) for 10,000 yr following the single ice-melting incident. For the North Sea, New York, and Hawaii, the sea level against time curves are similar in form, although slightly different in magnitude. Immediate submergence exceeding 100% of S is followed by slow emergence of only a few per cent of S . The predicted change in sea level on New Zealand is different because slow submergence, rather than emergence, occurs after the initial rapid rise of sea level. After 2,500 yr New Zealand also emerges slightly. Sea level falls at Cape Horn and the Ross Ice Shelf until 1,100 yr after the ice melting episode, then dramatic submergence occurs, drowning the present day beaches after 1,500 yr (at Cape Horn) and 3,400 yr (near the Ross Ice Shelf). Regions now under WIS will witness a continuing fall in sea level reaching magnitudes of up to -700% of S .

Conclusions

Sea level changes corresponding to future changes in the West Antarctic ice sheet will be affected by (1) changes in gravitational attraction exerted by the ice sheet on the surrounding ocean, (2) the

Earth's immediate elastic response to changed ice and water loads, and (3) the Earth's long-term response due to viscous flow within the mantle. If a uniform amount of ice was removed from WIS, the combined effect of these three processes would result in a non-uniform sea level change immediately afterwards ranging from +125% of the average global rise at Hawaii to -40% of the average rise near the Ross Ice Shelf, Antarctica.

Acknowledgment is made to the National Center for Atmospheric Research (NCAR), which is sponsored by the National Science Foundation, for computer time used in this research. The work was supported by the Division of Earth Sciences National Science Foundation, NSF Grants DES74-13047-A01 and EAR74-13047-A02. Thanks are extended to John T. Hollin for

critically reviewing the manuscript, and to W. Richard Peltier for supplying the necessary Green functions.

Received 4 May; accepted 30 June 1977.

- ¹ Farrell, W. E. & Clark, J. A. *Geophys. J. R. astr. Soc.* **46**, 647-667 (1976).
- ² Bentley, C. R. & Ostenson, N. A. *J. Glaciol.* **3**, 882-912 (1961).
- ³ Weertman, J. *J. Glaciol.* **13**, 3-11 (1974).
- ⁴ Hughes, T. J. *geophys. Res.* **78**, 7884-7910 (1973); *Rev. geophys. Space Phys.* **13**, 502-526 (1975); *Rev. geophys. Space Phys.* **15**, 1-46 (1977).
- ⁵ Denton, G. H. & Borns, H. W. Jr. *Antarct. J.* **9**, 167 (1974).
- ⁶ Denton, G. H., Borns, H. W. Jr., Grosswald, M. G., Stuiver, M. & Nichols, R. L. *Antarct. J.* **10**, 160-164 (1975).
- ⁷ Thomas, R. H. *Nature* **259**, 180-183 (1976).
- ⁸ Peltier, W. R. *Rev. geophys. Space Phys.* **12**, 649-669 (1974).
- ⁹ Cathies, L. M. *The Viscosity of the Earth's Mantle* 386 (Princeton University, Princeton, 1975).
- ¹⁰ Peltier, W. R. & Andrews, J. T. *Geophys. J. R. astr. Soc.* **46**, 605-646 (1976).
- ¹¹ Farrell, W. E. *Rev. geophys. Space Phys.* **10**, 761-797 (1972).
- ¹² Woodward, R. S. *U.S. geol. Survey Bull.* **48**, 87-170 (1888).
- ¹³ Clark, J. A. *Geology* **4**, 310-312 (1976).

Isotopic evidence for source of diagenetic carbonates formed during burial of organic-rich sediments

Hilary Irwin & Charles Curtis

Department of Geology, The University, Sheffield, UK

Max Coleman

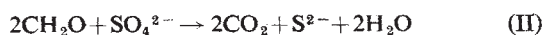
Institute of Geological Sciences, Geochemical Division, 64-78 Gray's Inn Road, London, UK

Organic matter is modified by several processes operating at different depths during burial diagenesis: (1) sulphate reduction; (2) fermentation; (3) thermally-induced decarboxylation, and so on. CO₂, one common product of each, can be distinguished by its carbon isotope composition: approximately (1) -25‰, (2) +15‰, (3) -20‰ relative to PDB. These values are preserved in diagenetic carbonates of the Upper Jurassic Kimmeridge Clay. Independent corroboration of the relative dominance of each process within specific depth intervals is given by the isotopic composition of incorporated oxygen which is temperature dependent (1) 0 to -2‰, (2) -1.5 to -5‰, (3) -3.5 to -7.0‰.

A BELATED recognition of the potentially disastrous supply/demand situation for liquid hydrocarbons has reawakened interest in organic matter maturation processes in sediments. Much emphasis is placed on thermally-induced reactions but little on several other processes which equally affect source rock potential. These are more important at shallower burial depths and also lead to diagenetic cementation which can affect both hydrocarbon migration and reservoir properties.

Curtis recently¹ proposed that several different depth-related zones could be recognised within burial sequences and that kinetic controls (notably rate of burial) dictated the relative importance of each zone in determining the extent and style of diagenetic modification.

Figure 1 represents an order-of-magnitude model for depth zonation with particular reference to the generation of carbon dioxide. Note the nonlinear depth scale which exaggerates the depth span of shallower zones. Bacterial processes dominate in zones I, II and III. Carbon dioxide is one product of organic matter degradation in each with the following equations indicating the nature of each overall reaction:



The boundary between zones I and II is determined by the extent of downward diffusion of dissolved molecular oxygen from overlying waters and the lower limit of zone II by downward diffusion of sulphate (we shall here restrict the discussion to fairly rapidly deposited marine mudstones containing abundant organic matter and within which sulphate reduction almost invariably occurs). Fermentation starts as soon as sulphate reduction stops (possibly because the bacteria cannot tolerate dissolved sulphides) and presumably continues until prevented by either high temperatures or exhaustion of suitable organic substrate. The rate of production of carbon dioxide within each zone decreases with depth due to diminishing oxygen, sulphate and substrate quality respectively.

Zone IV is less clearly defined with respect to depth or process. One well documented reaction is decarboxylation:



The rate of this (and similar) abiotic reaction presumably increases with rising temperature and then slows as suitable molecules are exhausted. Oxidation by detrital ferric compounds also must cause organic matter degradation: in Fig. 1 this is depicted to be relatively important at shallow depths where pyrite forms from reactive soil sesquioxides and again at greater depths where higher temperatures promote reduction of less reactive detrital phases.

The carbon dioxide produced by all these reactions dissolves readily in porewater to increase bicarbonate concentrations. Its carbon isotopic composition, however, must be anticipated to be very different from that of marine reservoir bicarbonate (~ 0‰PDB) since the source in each case is organic matter (~ -25‰PDB). Bacterial oxidation and sulphate reduction seem to impose little fractionation such that very light bicarbonate is to be anticipated. There is some evidence² for believing that decarboxylation produces somewhat heavier (but still light) carbon dioxide. We have found no information relating to fractionation during oxidation by ferric compounds and have assumed that none occurs. Fermentation, however, imposes an extremely large fractionation³ with bacterial meth-

ane values of -75‰_{PDB} commonplace⁴. Consequently, carbon dioxide produced in this reaction must be heavy and, in the absence of accurate information, we estimate something of the order of $+15\text{‰}$; certainly positive.

As sediments are buried, therefore, they pass successively through different zones within which organic matter is being altered and carbon dioxide produced. Bicarbonate activities sufficient to cause carbonate super-saturation are unlikely to be reached in zone I because of upward diffusion into depositional waters, but below this zone these levels are probable. The interesting feature is that carbonate precipitated from zone II should

be very light. There should then be a rapid and dramatic change to very heavy carbonates in zone III and then a less dramatic return to lighter values as fermentation becomes less important and decarboxylation more important into zone IV. It should be noted that unstable primary carbonates ($\delta^{13}\text{C} \sim 0\text{‰}_{\text{PDB}}$) might dissolve and add carbon of intermediate isotopic composition to the porewater reservoir in which case the above trends might be blurred.

Kimmeridge Clay

The onset of Kimmeridge Clay sedimentation marks a period of

Table 1 Isotopic composition and inferred temperatures and depth of precipitation, Kimmeridge carbonates

Sample description	No.	$\delta^{18}\text{O}_{\text{PDB}}$	$\delta^{13}\text{C}_{\text{PDB}}$	$T(^{\circ}\text{C})^*$	$T_d(^{\circ}\text{C})^\dagger$	Depth (m) ‡
'Primary' coccolithic limestones						
Coccolith-rich carbonates	846	-2.88	-0.35	24.3		—
	862	-2.92	-0.48	24.5		—
	875	-2.60	-0.56	23.0		—
	879	-3.52	-0.45	27.4		—
	881	-3.17	+0.28	25.7		—
	883	-3.28	-0.50	26.2		—
	887	-2.99	-0.57	24.8		—
	895	-3.45	-0.35	27.0		—
	896	-3.65	+0.08	28.0		—
	897	-3.73	+0.65	28.3		—
	898	-3.39	+0.39	26.7		—
	948	-3.47	-0.25	27.1		—
	953	-3.42	-0.60	26.8		—
	955	-3.14	+0.84	25.6		—
	997	-3.56	-2.21	27.5		—
	998	-3.85	-0.85	28.9		—
Diagenetic calcite nodules						
Rotunda nodule	1001	-0.74	-15.12	15.0		
	1002M	-1.46	-15.02	18.0		
Rotunda nodule	1002B	-0.17	-15.27	12.7		10 ‡
	817T	0.80	-17.50	15.2		
Blackstone nodule	817M	-1.28	-15.74	17.2		
	817B	-2.91	-14.57	24.5	?	?
Mean 15.6 (excluding 817B)						
Dolomitic/ankeritic cementstones						
	915	-3.30	+0.32	44.3	23.4	290
	911	-4.05	-0.86	49.1	27.3	430
	909	-3.56	+0.52	46.0	24.7	335
	903	-3.59	-0.97	46.1	24.9	340
	897	-4.54	-2.16	52.3	29.9	520
	866	-3.32	-0.53	44.4	23.5	290
	865	-3.78	-1.08	47.4	25.9	380
	853	-3.35	+0.59	44.6	23.7	300
'Basalt Stone' band	852	-2.99	+1.32	42.4	21.9	235
	851	-4.22	-2.68	50.2	28.2	460
	749	-4.73	-3.02	53.6	30.9	555
'Grey Ledge' band	748	-3.80	+1.79	47.5	26.0	380
	747	-4.64	-3.53	53.0	30.4	540
	699	-2.17	+6.20	37.4	18.0	95
'Cattle Ledge' band	698	-2.87	+5.44	41.6	21.3	215
	697	-4.81	-4.21	54.2	31.4	575
	649	-3.38	+2.30	44.8	23.8	395
'Yellow Ledge' band	648	-1.64	+9.28	34.4	15.6	10
	647	-4.79	-3.59	54.0	31.2	565
	604	-5.70	-4.42	60.3	36.4	755
	603	-5.79	-5.54	61.0	36.9	770
	602	-5.35	-4.16	57.9	34.4	680
	599	-6.42	-5.46	65.5	40.7	905
'Nine Inch' band	598	-4.97	-6.38	55.3	32.2	605
	549	-6.42	-5.31	65.5	40.7	905
'Maple Ledge' band	548	-5.50	-3.03	58.9	35.2	710
	547	-6.28	-5.09	64.5	39.8	875
	499	-5.83	-2.06	61.3	37.2	780
'Washing Ledge' band	498	-6.46	-4.21	65.8	40.9	915
	497	-4.83	-0.88	54.3	31.5	580
	449	-2.66	+7.96	40.4	20.3	180
'Flat Stone' band	448	-3.13	+6.14	43.3	22.6	260
	447	-6.08	-4.47	63.1	38.6	830

* T calculated for water, $\delta^{18}\text{O} = -1.2\text{‰}$.

$^\dagger T_d$ calculated for water, $\delta^{18}\text{O} = -4.9\text{‰}$.

‡ Depth values calculated assuming: temperature at base of sulphate reduction zone 15.6°C . Depth of base of sulphate reduction zone 10 m. Thermal gradient 28°C per km. No values given for primary (surface water) precipitation carbonates.

marine transgression and water deepening over much of Western Europe. This has been linked with widespread tectonically-induced subsidence⁵. The sediments consist of uniform clays and shales, mostly organic rich, with occasional cementstones (dolomitic calcilutites) and siltstones. The Dorset Coast section is some 500 m thick and reflects a relatively deep basinal area within widespread epicontinental seas. The section is generally organic rich and a few horizons have been mined as oil shales. The Kimmeridge Clay is widely believed to be the principle source rock for North Sea oil.

Cementstone horizons of great lateral persistence are common in the Dorset Coast section, especially in its lower part. Many of these have been given local names⁶ which are included in Table 1, column 1. Discrete diagenetic carbonate nodules also occur and these too were sampled. In the upper part of the succession a third distinctive carbonate sediment is found as bands of impure (organic-rich) chalk, referred to as 'coccolithic limestones'. In all, 57 carbonate-rich samples were taken. Sample numbers (Table 1, column 2) reflect relative position within the sequence.

This sequence is entirely marine, relatively rapidly deposited, organic rich and contains much carbonate, some of which is undoubtedly of diagenetic origin. It also is sulphur rich (diagenetic pyrite and sulphur in organic compounds). It therefore should be a good place to test the reality of the zonal model outlined in Fig. 1.

Experimental procedures

Thin sections (very thin in the case of particularly fine-grained rocks) were prepared from each carbonate sample and 20–50 g crushed for analysis. Chemical (after Pearson⁷) and X-ray diffraction data were obtained for most samples and these results will be discussed in detail elsewhere.

The vast majority of samples fall into just three classes. The cementstones consist mostly of fine-grained mosaics of interlocking crystals euhedral to subhedral in outline. The carbonate phase ranges in composition between $(\text{Ca}_{0.548} \text{Mg}_{0.336} \text{Fe}_{0.117})\text{CO}_3$ and $(\text{Ca}_{0.554} \text{Mg}_{0.365} \text{Fe}_{0.082})\text{CO}_3$. Strictly, the iron-rich samples are ankerites, and the iron-poor samples ferroan dolomites. d_{104} varies between 2.898 Å and 2.909 Å. For simplicity this phase will be referred to as dolomite below.

The coccolithic limestones are all fairly pure calcites, ranging between $(\text{Ca}_{0.979} \text{Mg}_{0.002} \text{Fe}_{0.020})\text{CO}_3$ and $(\text{Ca}_{0.989} \text{Mg}_{0.004} \text{Fe}_{0.007})\text{CO}_3$ with d_{104} between 3.032 Å and 3.035 Å. In thin section, elongated lenses of coccoliths are separated by organic-rich laminae. Bioturbation and small scale cross lamination (ripples?) are not uncommon.

The nodules consist of fine-grained microsparite calcite (d_{104} 3.0206 Å to 3.0213 Å). Considerable Mg^{2+} and/or Fe^{2+} substitution is indicated. Pyrite is common in the Blackstone nodules which also show late calcite veins (817 V). Rotunda nodules often enclose ammonites.

For isotopic analysis, carbonate powder samples were washed with 1% sodium hypochlorite solution⁸ to remove organic compounds which can interfere with accurate isotope ratio determinations⁹. Carbon dioxide was prepared from about 10 mg of the treated sample by reaction with 100% phosphoric acid at 25.0 °C using a method similar to that described by McCrea¹⁰. Pure calcite samples had reacted completely in 2 h whereas dolomites required about 36 h.

Carbon dioxide was analysed with a Micromass 602-C mass spectrometer and the raw data corrected using the methods of Craig¹¹ and Deines¹². All samples were analysed with reference to carbon dioxide from a calcite standard prepared at the same time. Consequently, oxygen isotope ratios for dolomites were recalculated to compensate for a different fractionation factor for acid decomposition (assumed to be that for pure dolomite, 1.01110, ref. 13). The appropriate factor for calcite is 1.01025. Data for both oxygen and carbon are presented in the normal δ notation with reference to PDB standard in Table 1, columns 3 and 4.

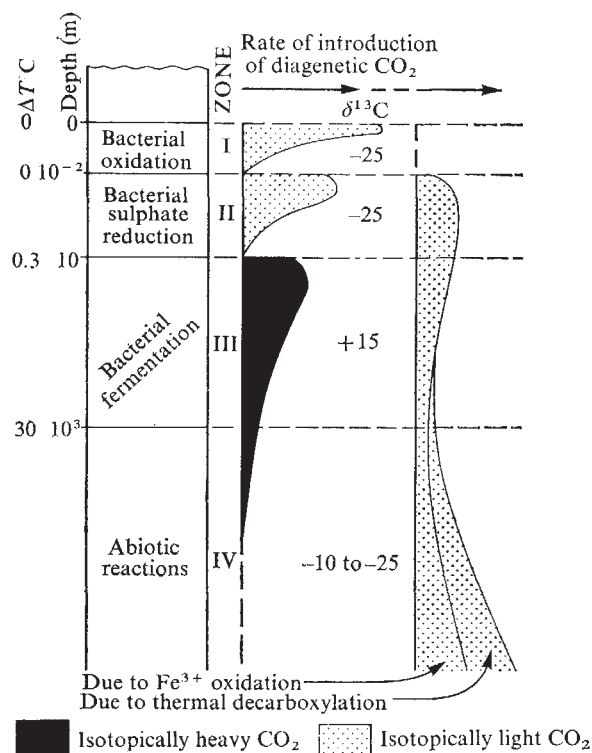


Fig. 1 Introduction of diagenetic CO_2 within different diagenetic zones.

The oxygen isotopic equilibrium fractionation between a carbonate and water is a function of temperature. For calcite the relationship is given by

$$T = 16.9 - 4.21 (\delta_c - \delta_w) + 0.14 (\delta_c - \delta_w)^2$$

(ref. 14) where $(\delta_c - \delta_w)$ is the measured difference in $\delta^{18}\text{O}$ between calcite and water and is used here in the same sense as originally defined by Epstein *et al.*¹⁶. But there is much controversy about the precise extent of the fractionation between dolomite and water. The extrapolation of high temperature experimental data^{16,17} to 25 °C suggests that dolomite should be 5–7‰ heavier than coexisting calcite. This contrasts with some measurements which show that coexisting sedimentary dolomites and calcites have similar isotopic values^{18,19}. Low temperature experimental precipitation of protodolomite¹⁹ shows that it should be approximately 3‰ heavier than coexisting calcite. This value is intermediate between other estimates and can be considered to correspond reasonably to the probable conditions of precipitation of Kimmeridge dolomites. The expression given below is calculated from the original data

$$T = 31.9 - 5.55 (\delta_d - \delta_w) + 0.17 (\delta_d - \delta_w)^2$$

No account has been taken of possible slight isotopic fractionations due to iron substitution in the dolomite lattice.

An isotopic composition of -1.20‰ , the value for pre-glacial oceans (Shackleton and Kennett²⁰) has been assumed for both seawater and porewater. The isotopic equilibrium temperature, T , for each sample is listed in Table 1, column 5.

Discussion

Figure 2 is a plot of $\delta^{18}\text{O}_{\text{PDB}}$ against $\delta^{13}\text{C}_{\text{PDB}}$. Two precipitation temperature scales are given, one for calcite and one for dolomite (cementstones). The isotopic composition of the coccolith limestones is very much that to be expected for precipitation from marine reservoir bicarbonate in warm

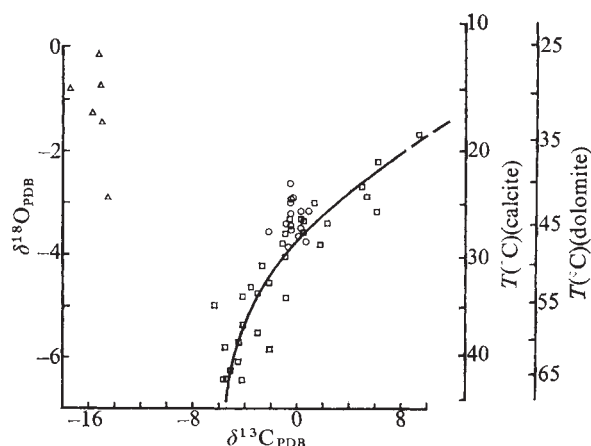


Fig. 2 Plot of oxygen isotope ratio against carbon isotope ratio for all samples. Triangles represent diagenetic calcites, squares diagenetic dolomites and circles coccolithic calcites. The two temperature scales relate to different fractionations of dolomite and calcite with water of $\delta^{18}\text{O} = -1.2\text{‰}$.

surface waters ($\delta^{13}\text{C} \sim \text{zero}$, $\delta^{18}\text{O} \sim -3.3\text{‰}$ corresponding to approximately 26°C). Bearing in mind that some of the samples contain minor diagenetic carbonate, the range is small.

The nodular calcites show very different isotope ratios with $\delta^{13}\text{C} \sim -16\text{‰}$ and $\delta^{18}\text{O} -0.8\text{‰}$ corresponding to a much lower precipitation temperature $T \sim 15^\circ\text{C}$ (exception sample 817B). These values lie well outside the range of all other samples. It seems reasonable to suggest that they precipitated not far below the sediment/water interface largely from carbon dioxide produced by sulphate-reducing bacteria (zone II, Fig. 1). The involvement of these bacteria during concretion development has been discussed by Raiswell²¹. The thermal gradient within marine depositional waters is in the reverse direction to that within buried sediments: the sediment/water interface must lie close to the temperature minimum within the total water plus sediment column. The presence of pyrite in these samples has already been noted.

The cementstones are remarkable for their wide range of both carbon and oxygen isotopic ratios. Carbon ranges from very heavy to light and oxygen isotope ratios indicate precipitation temperatures from 34°C to 66°C . Most remarkable of all is the very strong correlation between the two sets of data: heaviest carbonates being precipitated at the lowest temperatures. The obvious first interpretation is that these carbonates started to precipitate at some depth below the sediment/water interface from porewaters supersaturated with ^{13}C -rich bicarbonate and that precipitation continued to much greater burial depths where porewater bicarbonate was very much poorer in ^{13}C , in fact enriched in ^{12}C relative to marine reservoir bicarbonate. All these data can be matched easily with the zonal model in Fig. 1. The lower temperature dolomite samples precipitated near the top of zone III where fermentation processes introduced ^{13}C -rich bicarbonate from organic matter. The gradual and systematic changeover to ^{12}C -rich carbonates with depth of precipitation reflects the increasing importance of abiotic oxidation and decarboxylation reactions relative to fermentation downwards through zone III into zone IV. Although some cementstones contain minor calcite and it is possible that some primary calcite dissolved to contribute bicarbonate to the porewater pool, the observed trend is so well developed that we are forced to conclude that bicarbonate derived from organic matter was the principle source for both cementstones and nodular calcites.

From this discussion we feel that the isotope data obtained strongly support the view that distinctive depth-related zones of diagenesis, as shown in Fig. 1, persisted within the sediment column during Kimmeridge times. This work underlines the importance of diagenetic reactions being responsible for

significant modification of organic matter and the production of massive amounts of carbonate cement. There remains the intriguing and possibly important prospect of being able to determine the depth of precipitation assuming a particular thermal gradient.

The precipitation temperature data (Table 1, column 5 with the exception of Sample 817B) indicate a considerable break between zones II and III. For a thermal gradient of about 30°C per km this implies that bacterial fermentation first becomes important at depths of the order of 700 m. This is at variance with the proposition that the onset of fermentation is simply precluded by the activities of sulphate-reducing bacteria. There are, however, at least two reasons for doubting the reality of this large break between zones II and III.

As mentioned above, there is some uncertainty in the calibration of dolomite/water fractionation as a function of temperature (especially as the effect of iron substitution is unknown). If, for example, the value for dolomite were the same as that for calcite, as suggested by some evidence^{18,19} then the break would not exist. Using experimentally-derived calibrations^{16,17} would, of course, increase its magnitude. We do not think, however, that calibration uncertainties alone could account for the scale of the temperature/depth break.

There is a more compelling argument for doubting the reality of the break which also reconciles experimental and geological evidence. The major isotopic effect of diagenesis on porewater is depletion of ^{18}O associated with formation of diagenetic minerals enriched in that isotope. This applies to clay minerals as well as carbonates. The main accompanying physical effect is reduction of pore space and upward expression of water. At depth one would expect diagenetic minerals to be formed in equilibrium with isotopically light modified connate water. Measurements on porewater in samples recovered from the Deep Sea Drilling Project^{22,23} show a systematic reduction of $\delta^{18}\text{O}$ with depth to a maximum of 3‰ at 300 m. This was attributed to diagenetic reactions. This depletion should prevail at all depths below a mixing zone open to the effectively infinite seawater reservoir. The Kimmeridge sediments were probably deposited much more rapidly than those referred to above such that the 'mixing zone' was relatively limited in extent and effective for a shorter time with respect to any individual sediment unit. It should be noted that enrichment of ^{18}O in formation waters by exchange with isotopically heavy rocks²⁴ is a quite different effect from that produced by precipitation of diagenetic minerals.

A useful if very simple model which incorporates these effects can be constructed if certain assumptions are allowed. The downward limit of sulphate reduction is controlled by diffusion (see above): this limit thus crudely approximates to the boundary between open access to depositional water above and a closed system below. If we assume that the lowest temperature (shallowest) dolomite sample (No. 648) formed immediately below the sulphate reduction zone, then it would have precipitated at a similar temperature to nodular calcite (average 15.6°C). The $\delta^{18}\text{O}$ value of water in equilibrium with dolomite at this temperature is -4.9‰ . This figure can be thought of as the net depletion, caused by diagenetic processes in the burial column. To simplify the calculation we assume that the expressed water maintained this value throughout the uppermost 1 km of the sediment column and then changed abruptly to -1.2‰ at the sulphate-reduction zone.

Precipitation temperatures for the deeper diagenetic carbonates were calculated on this basis. These values, T_d , are given in Table 1, column 6. This approach, of course, is a gross oversimplification since there will be variation in $\delta^{18}\text{O}$ with depth below the mixing zone boundary. It nevertheless represents a reasonable first approximation. By assuming the temperature of the base of the sulphate-reduction zone is 15.6°C , its depth to be 10 m and a thermal gradient of 28°C per km (a value used in ref. 1), precipitation depths can be calculated and these are listed in Table 1, column 7. In Fig. 3, the various carbonate samples are plotted as $\delta^{13}\text{C}$ against precipitation temperature:

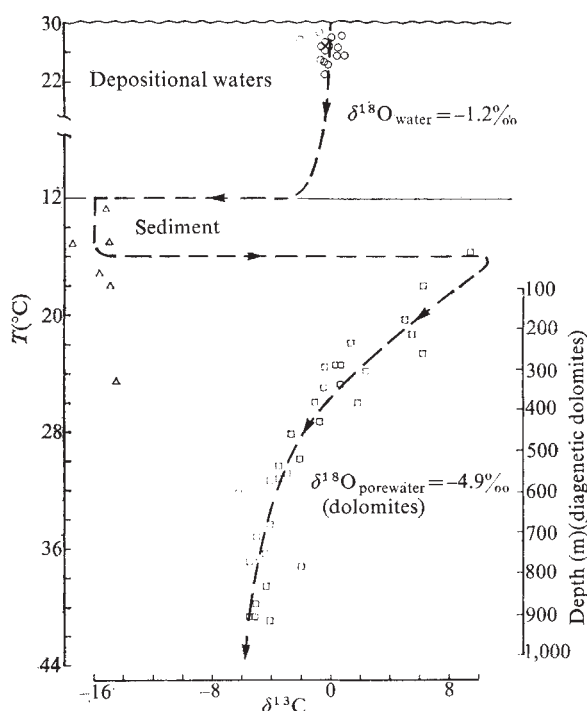


Fig. 3 Plot of calculated precipitation temperatures for Kimmeridge carbonates against carbon isotope ratio. 'Primary' carbonates are shown associated with depositional waters, diagenetic carbonates with sediment porewaters. Precipitation pathway is indicated showing passage through zone II (sulphate reduction) to zone III (fermentation-heavy C) to zone IV at the base where light carbon isotope ratios are again encountered. For discussion of porewater isotope composition and precipitation depth estimates see text. Symbols as for Fig. 2.

T for calcites, T_d for dolomites. Coccolithic limestone samples are plotted separately since they formed within the depositional environment.

Estimates of burial depths for precipitation of diagenetic dolomites suggest values ranging to 1 km. This means that all these carbonates precipitated relatively early during diagenesis and almost certainly predated liquid hydrocarbon formation or migration. The trend depicted in Fig. 3 represents a time

sequence for precipitation of carbonates at any particular sediment horizon. Carbonates precipitated from depositional waters obviously constitute the first component. Shortly after burial sulphate reduction may promote precipitation of early diagenetic carbonate, very rich in ^{12}C and reflecting low temperatures. The next carbonates to form will do so at similar temperatures but will have extremely different carbon isotope ratios with ^{13}C enrichment in consequence of fermentation reactions. Thereafter, successively later carbonates will reflect higher precipitation temperatures and the increasing influence of abiotic and decarboxylation reactions (^{12}C -rich bicarbonate input).

Work in hand involves extension of the investigation to later (deeper) precipitated carbonates in sandstones as well as in mudstone sequences. It is hoped to improve the calibration of depth/temperature scales as better models and new data become available.

We thank Professor C. Downie for help and advice, and Dr J. D. Hudson (Leicester University) for constructive criticism. Isotopic measurements were made in the Institute of Geological Sciences. The Natural Environment Research Council is supporting this work through a Research Studentship (H.I.) and Research Grant GR3-3086 (C.D.C.).

Received 17 March; accepted 20 June 1977.

- ¹ Curtis, C. D. *Phil. Trans. R. Soc.* (in the press).
- ² Abelson, P. & Hoering, T. C. *Proc. natn. Acad. Sci. U.S.A.* **47**, 623 (1961).
- ³ Rosenfeld, W. & Silverman, S. *Science* **130**, 1658 (1959).
- ⁴ Claypool, G., Presley, B. J. & Kaplan, I. R. in *Initial Reports of the Deep Sea Drilling Project* 19, 879 (US Government Printing Office, Washington, D.C., 1973).
- ⁵ Hallam, A. & Sellwood, B. J. *J. Geol.* **84**, 301 (1976).
- ⁶ Arkell, W. J. *The Jurassic System of Great Britain* (Oxford University Press, Oxford, 1933).
- ⁷ Pearson, M. J. *Miner. Mag.* **39**, 696 (1974).
- ⁸ Forester, R. H., Sandberg, P. A. & Anderson, T. F. in *Living and Fossil Bryozoa* (ed. Larwood, G. P.) (Academic, London and New York, 1973).
- ⁹ Weber, J. N., Deines, P., Weber, P. H. & Baker, P. A. *Geochim. cosmochim. Acta* **40**, 31 (1976).
- ¹⁰ McCrea, J. M. *J. chem. Phys.* **18**, 849 (1950).
- ¹¹ Craig, H. *Geochim. cosmochim. Acta* **12**, 133 (1957).
- ¹² Deines, P. *Int. J. Mass Spectrom. Ion Phys.* **4**, 283 (1970).
- ¹³ Sharma, T. & Clayton, R. N. *Geochim. cosmochim. Acta* **29**, 1317 (1965).
- ¹⁴ Craig, H. *Proc. Spoleto Conf. on Stable Isotopes in Oceanographic Studies and Paleotemperatures*, 3 (1965).
- ¹⁵ Epstein, S., Buchsbaum, H. A., Lowenstam, H. A. & Urey, H. C. *Bull. geol. Soc. Am.* **64**, 1315 (1953).
- ¹⁶ Northrop, D. A. & Clayton, R. N. *J. Geol.* **74**, 174 (1966).
- ¹⁷ O'Neil, J. R. & Epstein, S. *Science* **152**, 198 (1966).
- ¹⁸ Degens, E. T. & Epstein, S. *Geochim. cosmochim. Acta* **28**, 23 (1964).
- ¹⁹ Fritz, P. & Smith, D. G. W. *Geochim. cosmochim. Acta* **34**, 1161 (1970).
- ²⁰ Shackleton, N. J. & Kennett, J. P. in *Initial Reports of the Deep Sea Drilling Project* 29, 743 (US Government Printing Office, Washington, D.C., 1975).
- ²¹ Raiswell, R. W. *Chem. Geol.* **18**, 227 (1976).
- ²² Lawrence, J. R., Gieskes, J. M. & Broecker, W. S. *Earth planet. Sci. Lett.* **27**, 1 (1975).
- ²³ Perry, E. A., Jr., Gieskes, J. M. & Lawrence, J. R. *Geochim. cosmochim. Acta* **40**, 413 (1976).
- ²⁴ Clayton, R. N. *J. geophys. Res.* **71**, 389 (1966).

X-ray structures of two oxidation states of a flavin-nicotinamide biscoenzyme and models for flavin—nicotinamide interactions

David J. T. Porter* & Harold J. Bright

Department of Biochemistry and Biophysics, University of Pennsylvania, Philadelphia, Pennsylvania 19104

Donald Voet†

Department of Chemistry and Laboratory for Research on the Structure of Matter, University of Pennsylvania, Philadelphia, Pennsylvania 19104

The flavin nicotinamide biscoenzymes $\text{Fl}_{\text{ox}}^- - \text{C}_3 - \text{Nic}^+$ and $\text{H}_2\text{Fl}_{\text{red}} - \text{C}_3 - \text{Nic}^+$ assume extended conformations in the solid state. In both derivatives the nicotinamide and flavin groups associate through hydrogen bonding. The bending angle of the reduced flavin moiety is less than half that in any previously reported 1,5-dihydroflavin structure. This effect is apparently due to ring stacking interactions.

REDUCTION of a flavoenzyme by NADH is the primary step in many important biological oxidoreduction processes. Interactions between flavin and nicotinamide species of differing oxidation states have been described in several such flavoenzyme catalysed

*Present address: Novo Laboratories, 59 Danbury Road, Wilton, Connecticut.

†To whom correspondence should be addressed.

reactions¹. Complexes composed of reduced flavin and oxidised nicotinamide have been characterised as charge-transfer complexes by spectral criteria² as well as by the correlation of the energies of their long wavelength absorptions with the calculated lowest vacant orbital energies of their nicotinamide components³. Similarly, kinetic and spectral data have indicated that a kinetically important charge-transfer complex between reduced nicotinamide and oxidised flavin occurs before the reduction of flavins⁴⁻⁸. The geometry of this complex has been proposed to be one in which the two ring systems are face-to-face such that the nicotinamide C(4) atom is opposite the flavin N(5) atom⁵. Flavin-nicotinamide oxidoreduction is then postulated to occur by hydride ion transfer from the nicotinamide C(4) position to the flavin N(5) position⁵. Alternately, it has been proposed that such reactions occur through a one electron transfer followed by the migration of a hydrogen atom⁹. Chemical evidence supporting these mechanisms is provided by the demonstration of direct hydrogen transfer from dihydronicotinamide to the C(5) position of N(5)-deazaflavin, both in a model system¹⁰ and enzymatically¹¹.

This study was undertaken in order to visualise the associations between nicotinamide and flavin in their various oxidation states. In doing so, we have elucidated the structures of two oxidation states of a propyl linked flavin-nicotinamide biscoenzyme, $\text{Fl}_{\text{ox}}^- - \text{C}_3 - \text{Nic}^+$ and $\text{H}_2\text{Fl}_{\text{red}} - \text{C}_3 - \text{Nic}^+$. $\text{Fl}_{\text{ox}}^- - \text{C}_3 - \text{Nic}^+$: 10-[3-(3-carbamoyl-1-pyridinium)-propyl]-7,8-dimethylisoalloxazine. $\text{Fl}_{\text{ox}}^- - \text{C}_3 - \text{Nic}^+$: The anion of $\text{Fl}_{\text{ox}} - \text{C}_3 - \text{Nic}^+$ generated by ionisation of the flavin N(3) position. $\text{H}_2\text{Fl}_{\text{red}} - \text{C}_3 - \text{Nic}^+$: 10-[3-(3-carbamoyl-1-pyridinium)-propyl]-1,5-dihydro-7,8-dimethylisoalloxazine. $\text{HFl}_{\text{red}} - \text{C}_3 - \text{Nic}^+$: The anion of $\text{H}_2\text{Fl}_{\text{red}} - \text{C}_3 - \text{Nic}^+$ generated by the ionisation of the flavin N(1) position. These compounds, which were originally synthesised for model studies of the 1,4-dihydronicotinamide reduction of flavins², exhibit intermolecular associations that are of potential biological significance.

Experimental methods

$\text{Fl}_{\text{ox}}^- - \text{C}_3 - \text{Nic}^+ \cdot 7\text{H}_2\text{O}$ synthesis: $(\text{Fl}_{\text{ox}} - \text{C}_3 - \text{Nic}^+)\text{Br}^-$ was synthesised as previously described^{2,5}. Needle shaped yellow crystals were grown by the vapour equilibration of a 10 mM aqueous NH_3 solution with a 5 mM $(\text{Fl}_{\text{ox}} - \text{C}_3 - \text{Nic}^+)\text{Br}^-$ solution. Thin layer chromatography of these crystals followed by staining with AgNO_3 failed to reveal the presence of bromide ion. Hence the

crystals consist of the zwitterionic species $\text{Fl}_{\text{ox}}^- - \text{C}_3 - \text{Nic}^+$. A crystal measuring $0.16 \times 0.13 \times 0.62$ mm was sealed inside a thin walled glass capillary tube to prevent the observed disintegration of the crystals on exposure to air. Oscillation and Weissenberg photographs revealed the space group to be $\text{P}2_1/\text{c}$. The unit cell parameters are $a = 7.740(3)$, $b = 23.800(12)$, $c = 13.958(5)$ Å and $\beta = 101.93(3)^\circ$. The crystal density of 1.392 g cm^{-3} , as determined by flotation in a CCl_4 -cyclohexane mixture, indicates that the contents of the asymmetric unit are $\text{Fl}_{\text{ox}}^- - \text{C}_3 - \text{Nic}^+ \cdot 7\text{H}_2\text{O}$.

$(\text{H}_2\text{Fl}_{\text{red}} - \text{C}_3 - \text{Nic}^+)\text{NO}_3^- \cdot 4\text{H}_2\text{O}$ synthesis: The flavin moiety in an aqueous solution of $(\text{Fl}_{\text{ox}} - \text{C}_3 - \text{Nic}^+)\text{Br}^-$ at pH 8.0 was selectively reduced by dithiothreitol in anaerobic conditions. This caused the solution to change colour from the yellow characteristic of oxidised flavins to dark blue-black. After a day the zwitterionic species $\text{HFl}_{\text{red}}^- - \text{C}_3 - \text{Nic}^+$ had precipitated as black spherical clumps of crystallites that were unsuitable for X-ray study. These were dissolved at about 50°C in a minimal amount of 50 mM HNO_3 to form a dark brown solution. After several days, red plate-like crystals appeared. These crystals are stable in air for several minutes but longer exposures result in their disintegration and the return of the yellow colour of oxidised flavins.

A crystal measuring $0.75 \times 0.08 \times 0.12$ mm was sealed inside a thin walled glass capillary tube together with a small amount of 0.1 M $\text{Na}_2\text{S}_2\text{O}_4$ to act as an oxygen scavenger. Oscillation, Weissenberg and precession photographs showed the crystal to have triclinic lattice symmetry. The statistical distribution of the subsequently measured diffraction data indicated that the space group of the crystal was $\text{P}1$. The unit cell parameters are $a = 8.068(3)$, $b = 11.369(3)$, $c = 14.190(7)$ Å, $\alpha = 77.89(3)$, $\beta = 88.99(4)$ and $\gamma = 89.12(3)^\circ$. The crystal density of 1.405 g cm^{-3} , as determined by flotation in a cyclohexane- CCl_4 mixture, indicated the stoichiometry of the asymmetric unit to be $(\text{H}_2\text{Fl}_{\text{red}} - \text{C}_3 - \text{Nic}^+)\text{NO}_3^- \cdot 4\text{H}_2\text{O}$.

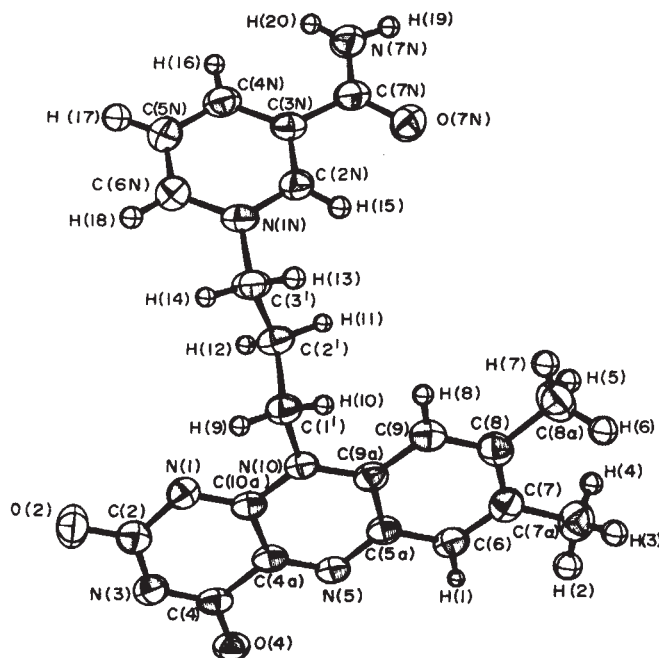
A detailed description of the techniques used in intensity measurement, data treatment and structure determination for both of these compounds will appear elsewhere¹². For both crystals, reflection intensities were measured by a Picker FACS-I diffractometer using graphite monochromatised $\text{CuK}\alpha$ radiation. A total of 3,806 and 4,076 reflections, respectively, for $\text{Fl}_{\text{ox}}^- - \text{C}_3 - \text{Nic}^+$ and $(\text{H}_2\text{Fl}_{\text{red}} - \text{C}_3 - \text{Nic}^+)\text{NO}_3^-$ were measured to the limit $2\theta = 125^\circ$. Both structures were solved by the straightforward application of the direct methods program MULTAN¹³. In both cases all 30 non-hydrogen atoms of the $\text{Fl} - \text{C}_3 - \text{Nic}^+$ skeleton appeared as the highest peaks in the E-map. Subsequent Fourier maps revealed the positions of the non-hydrogen atoms of seven water molecules in the case of $\text{Fl}_{\text{ox}}^- - \text{C}_3 - \text{Nic}^+$ and those of the nitrate ion and four water molecules for $(\text{H}_2\text{Fl}_{\text{red}} - \text{C}_3 - \text{Nic}^+)\text{NO}_3^-$.

The structures were refined by least-squares methods. Hydrogen atoms were located in difference Fourier maps and were subsequently also refined. Seven solvent hydrogen atoms could not be located in the $\text{Fl}_{\text{ox}}^- - \text{C}_3 - \text{Nic}^+$ structure and, similarly, four methyl hydrogens and two water hydrogens were not found in that of $(\text{H}_2\text{Fl}_{\text{red}} - \text{C}_3 - \text{Nic}^+)\text{NO}_3^-$. The refinements converged at the final agreement factors of $R = 0.064$ based on 3,318 observed reflections for $\text{Fl}_{\text{ox}}^- - \text{C}_3 - \text{Nic}^+$ and $R = 0.069$ based on 2,963 observed reflections for $(\text{H}_2\text{Fl}_{\text{red}} - \text{C}_3 - \text{Nic}^+)\text{NO}_3^-$.

Molecular structures

$\text{Fl}_{\text{ox}}^- - \text{C}_3 - \text{Nic}^+$: The final atomic parameters of the structures together with their covalent distances and angles will be presented elsewhere¹². Figure 1 illustrates the conformation of the $\text{Fl}_{\text{ox}}^- - \text{C}_3 - \text{Nic}^+$ zwitterion and presents the atomic numbering scheme. The covalent bonding parameters are mostly in excellent agreement with published values of corresponding distances and angles in similar molecules¹⁴⁻¹⁹. The only significant exceptions to this are the predictable distortions of the flavin pyrimidinoid ring in the region of the ionised N(3) atom^{14,20}. The flavin residue is nearly planar as has been found for other oxidised flavins². The root mean square deviation of its 18 atoms from coplanarity is 0.026 Å. That of

Fig. 1 A perspective drawing of the $\text{Fl}_{\text{ox}}^- - \text{C}_3 - \text{Nic}^+$ zwitterion. Atoms are shown as thermal ellipsoids.



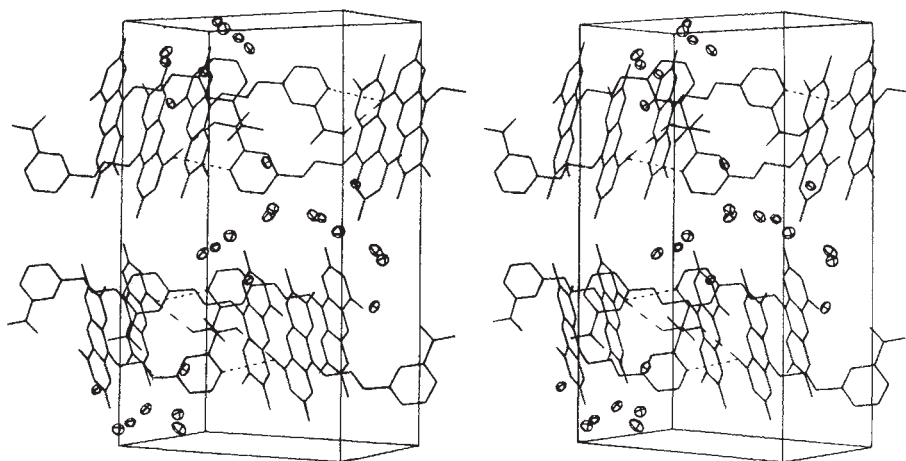


Fig. 2 A stereo diagram illustrating the packing of the $\text{Fl}_{\text{ox}}^{-}-\text{C}_3-\text{Nic}^{+}$ unit cell. The view is roughly along the a axis with the b and c axes extending vertically and horizontally, respectively. Water oxygen atoms are illustrated as thermal ellipsoids. Hydrogen bonds and $\text{C}-\text{H}\cdots\text{O}$ hydrogen bond-like interactions are shown as dashed and dotted lines, respectively. Hydrogen atoms have been omitted for clarity.

the 6 atoms of the nicotinamide ring is $0.005 \text{ \AA}$, so this ring is planar to within experimental error. The amide group is oriented such that the $\text{C}(7\text{N})-\text{N}(7\text{N})$ bond is *trans* to the $\text{N}(1\text{N})-\text{C}(3')$ bond with the planes of the amide group and the nicotinamide ring forming a dihedral angle of 1.1° . This nearly coplanar conformation is found in a majority of the known nicotinamide structures^{16-19,22}.

The propyl group is in the all *trans*, fully extended conformation. The least-squares planes through the nicotinamide and flavin rings form a dihedral angle of 59.5° .

Figure 2 is a stereo diagram showing the packing of the structure in the unit cell. The crystal structure largely consists of broad bands, endlessly extending parallel to the (010) plane, that contain the $\text{Fl}_{\text{ox}}^{-}-\text{C}_3-\text{Nic}^{+}$ zwitterions. Within these bands the flavin residues associate by forming endless stacks of overlapping parallel ring molecules. Here neighbouring molecules are related by centres of symmetry. The distances between the least-squares planes of the parallel flavin residues are 3.460 and $3.385 \text{ \AA}$, respectively, for the two different stacking interactions. There is no interatomic contact in either interaction that is less than its corresponding van der Waals distance. Hence these associations are normal stacking interactions.

The nicotinamide residues extend alternately to opposite sides of the flavin stacks. The nicotinamide rings within a given band of heterocycles are parallel due to symmetry considerations. As can be seen in Fig. 2, however, neighbouring nicotinamide residues do not overlap.

The only hydrogen atoms of the $\text{Fl}_{\text{ox}}^{-}-\text{C}_3-\text{Nic}^{+}$ zwitterion that are capable of forming normal hydrogen bonds are those of the amide group. Of these, only one participates in a hydrogen bond between the nicotinamide and flavin moieties of the structure. This is the intermolecular $\text{N}(7\text{N})-\text{H}(20)\cdots\text{O}(4)$ hydrogen bond illustrated in Figs 2 and 3. This interaction is flanked by an apparently strong intermolecular $\text{C}(4\text{N})-\text{H}(16)\cdots\text{O}(4)$ hydrogen bond-like interaction. Both of these interactions are unusual in that the nicotinamide and flavin planes are more nearly perpendicular than parallel to each other. Flavin atom $\text{N}(5)$, a potential hydrogen bond acceptor, is $2.86 \text{ \AA}$ from amide hydrogen $\text{H}(20)$. This is, however, too large a distance to be considered a hydrogen bond.

The bands containing the $\text{Fl}_{\text{ox}}^{-}-\text{C}_3-\text{Nic}^{+}$ zwitterions are interspersed by parallel bands containing the solvent molecules. These are arranged in a complex hydrogen bonded network which also involves the hydrophilic groups of the $\text{Fl}_{\text{ox}}^{-}-\text{C}_3-\text{Nic}^{+}$ zwitterion.

$\text{H}_2\text{Fl}_{\text{red}}-\text{C}_3-\text{Nic}^{+}$: Figure 4 illustrates the conformation of the $\text{H}_2\text{Fl}_{\text{red}}-\text{C}_3-\text{Nic}^{+}$ ion and the atomic numbering scheme. The covalent bonding parameters are in reasonable agreement with the corresponding values reported for other 1,5-dihydroisalloxazine derivatives.

The eight atoms of the pyrimidinoid group and the six atoms of the benzenoid ring of the flavin moiety deviate from their respective least-squares planes by root mean square distances of 0.006 and $0.012 \text{ \AA}$. Hence these rings are both highly planar. The reduced flavin nucleus assumes the expected 'butterfly' confor-

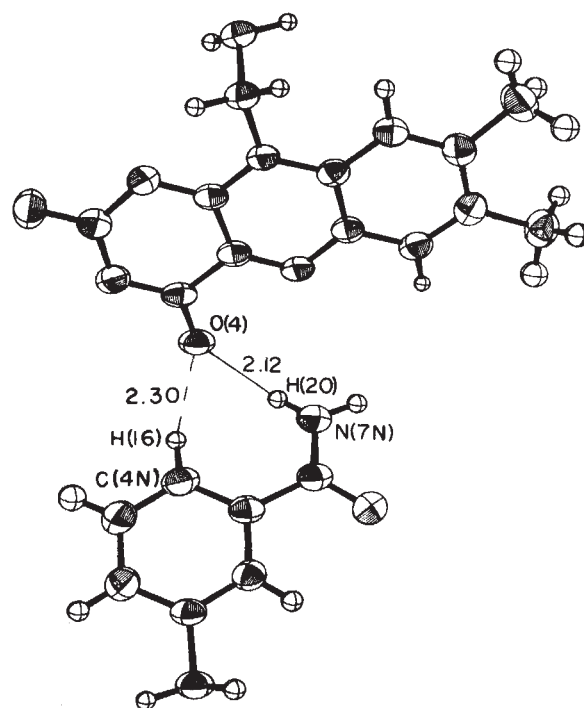
mation²³ with a 12.7° dihedral angle between the foregoing planes. The corresponding angles in the four previously reported 1,5-dihydroflavin structures range from 28.8 to 35.5° (refs 21, 24)—all more than twice that found in the present structure. The pyrazoid ring is in the usual boat conformation.

The nicotinamide ring is also highly planar. Its six atoms deviate from their least-squares plane by a root mean square distance of $0.008 \text{ \AA}$. The amide group in this ion is roughly *trans* to the $\text{N}(1\text{N})-\text{C}(3')$ bond but is twisted about the $\text{C}(3\text{N})-\text{C}(7\text{N})$ bond such that the $\text{C}(2\text{N})-\text{C}(3\text{N})-\text{C}(7\text{N})-\text{O}(7\text{N})$ torsion angle is 29.4° .

The propyl group is folded such that the conformation about its $\text{C}(2')-\text{C}(3')$ bond is *gauche* and that about its $\text{C}(1')-\text{C}(2')$ bond is *trans*. The dihedral angle between the least-squares planes through the nicotinamide ring and the entire flavin residue is 73.4° .

Figure 5 is a stereo diagram illustrating the packing of the structure in the unit cell. It can be seen that the flavins associate,

Fig. 3 A perspective drawing illustrating the hydrogen bonding interactions between the flavin and nicotinamide residues of neighbouring molecules in the structure of $\text{Fl}_{\text{ox}}^{-}-\text{C}_3-\text{Nic}^{+}$. Atoms are shown as thermal ellipsoids. Hydrogen bonds and $\text{C}-\text{H}\cdots\text{O}$ hydrogen bond-like interactions are represented by thin and dashed lines, respectively, and are accompanied by their corresponding lengths ($\text{\AA}$).



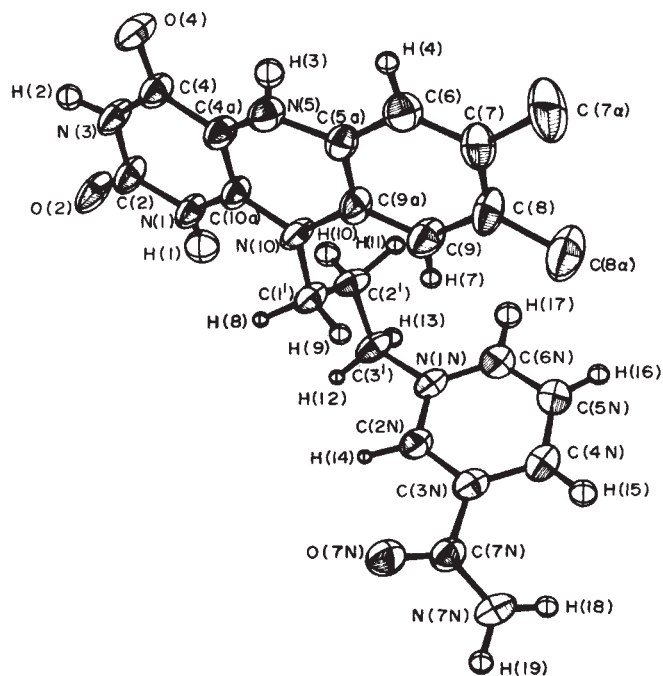


Fig. 4 A perspective drawing of the $\text{H}_2\text{Fl}_{\text{red}}\text{-C}_3\text{-Nic}^+$ ion. Atoms are shown as thermal ellipsoids.

through stacking and hydrogen bonding interactions, into bands of ring molecules endlessly extending parallel to the (001) plane. Neighbouring heterocycles within the columns of stacked flavins are related by centres of symmetry. Their butterfly conformation thereby causes them to alternately fold towards and away from each other. There are no interatomic contacts closer than van der Waals distances within these stacks.

Neighbouring flavins base pair across centres of symmetry in a manner by which flavins have often been observed to associate²⁵. The $\text{N}(3)\cdots\text{H}(2)\cdots\text{O}(2)$ hydrogen bond length of 2.796 Å is within the normal range for such interactions¹⁴.

The nicotinamide residues alternately extend to opposite sides of the flavin stacks and are oriented such that they are nearly parallel to the flavin stacking axis. The nicotinamide groups are too far removed from one another to associate in any manner.

The nicotinamide amide oxygen $\text{O}(7\text{N})$ accepts a tenuous hydrogen bond from the flavin $\text{N}(5)\text{-H}(3)$ group of a neighbouring $\text{H}_2\text{Fl}_{\text{red}}\text{-C}_3\text{-Nic}^+$ ion. This association, illustrated in Figs 5 and 6, seems to be quite strained. The $\text{N}(5)\cdots\text{O}(7\text{N})$ and the

$\text{H}(3)\cdots\text{O}(7\text{N})$ distances of 3.184 and 2.53 Å, respectively, are very long for $\text{N-H}\cdots\text{O}$ hydrogen bonds. The $\text{N}(5)\text{-H}(3)\cdots\text{O}(7)$ angle of 152° deviates significantly from the expected near linearity for such interactions. Furthermore, although an amide group is normally expected to be essentially coplanar with an N-H group to which it is hydrogen bonded, flavin atoms $\text{N}(5)$ and $\text{H}(3)$ deviate 2.399 and 1.98 Å, respectively, from the plane of the amide group with which they are associated. In fact, without the large $\text{C}(2\text{N})\text{-C}(3\text{N})\text{-C}(7\text{N})\text{-O}(7\text{N})$ torsional angle, the foregoing hydrogen bond could not be formed.

The regions in the crystal structure between the associating $\text{H}_2\text{Fl}_{\text{red}}\text{-C}_3\text{-Nic}^+$ ions are occupied by the nitrate ions and the solvent molecules. These associate through hydrogen bonded networks that involve the hydrophilic groups of the heterocycles. All potential hydrogen bonds in the structure seem to have been formed.

Discussion

Studies of the nitroalkane reduction of the FAD requiring enzyme D-amino acid oxidase have proven that, at least in certain classes of enzymatic reactions, the flavin $\text{N}(5)$ atom is the position through which electrons are transferred²⁶. This is further corroborated by the reduction of $\text{N}(5)$ -deazaflavin by direct hydrogen transfer to its $\text{C}(5)$ position^{10,11}. Thus it is of interest that the flavin $\text{N}(5)\text{-H}(3)$ group is hydrogen bonded to nicotinamide atom $\text{O}(7\text{N})$ in $\text{H}_2\text{Fl}_{\text{red}}\text{-C}_3\text{-Nic}^+$.

The $\text{C}(4)$ atom of nicotinamide is known to be the position through which electrons are transferred in the biological oxidation-reduction reactions of pyridine nucleotides. It is therefore of note that in $\text{Fl}_{\text{ox}}\text{-C}_3\text{-Nic}^+$ the $\text{C}(4\text{N})\text{-H}(16)$ group forms a hydrogen bond-like association with flavin atom $\text{O}(4)$.

The nicotinamide amide group has been shown not to be an essential component of NAD^+ in the reactions mediated by certain dehydrogenases²⁷. Rather, it seems that a major requirement for the activity of these enzymes is that the substituent to the pyridinium 3-position be a carbon atom that is double bonded to an electronegative atom such as O, N or S^{27} . Nevertheless, it seems likely that the amide group of nicotinamide serves a specific function in some vital biological process for without such a function it is expected that evolutionary processes would have chemically altered the amide group in some manner in at least some organisms. The similarities of the nicotinamide-flavin associations in both structures presented here suggest that the function of the nicotinamide amide group during enzyme mediated reduction of flavin by 1,4-dihyronicotinamide may be to bind the reactive portions of flavin and nicotinamide together to facilitate electron transfer.

The observed 12.7° bending angle of the 1,5-dihydroflavin

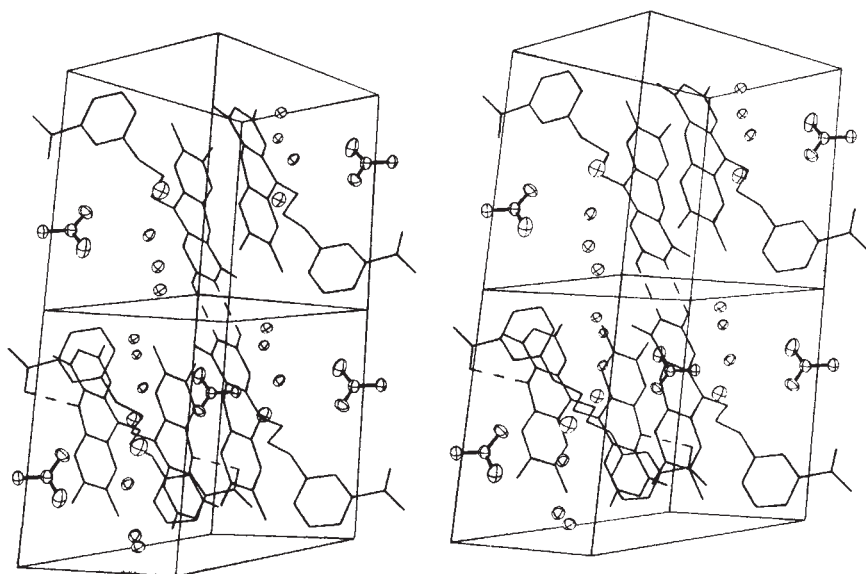


Fig. 5 A stereo drawing illustrating the crystal structure of $(\text{H}_2\text{Fl}_{\text{red}}\text{-C}_3\text{-Nic}^+)\text{NO}_3\cdot 4\text{H}_2\text{O}$ in relation to the unit cell boundaries. The view is roughly along the c axis with the a and b axes extending horizontally and vertically, respectively. Water oxygen atoms and the atoms of the nitrate ion are shown as thermal ellipsoids. Hydrogen bonds between heterocycles are shown as dashed lines. Hydrogen atoms have been omitted for clarity.

moiety of $\text{H}_2\text{Fl}_{\text{red}}\text{-C}_3\text{-Nic}^+$ is considerably less than the 32° average of this angle in the previously reported 1,5-dihydroflavin structures^{21,24}. All of these latter compounds have more and bulkier substituents than does the flavin residue in $\text{H}_2\text{Fl}_{\text{red}}\text{-C}_3\text{-Nic}^+$ and hence it might seem that their large bending angles are due to steric interference among these substituents. Molecular models of these compounds, however, reveal that such overcrowding is relieved at bending angles of the order of 10° . Furthermore, a similarly substituted fully oxidised flavin is observed to be nearly planar²⁸.

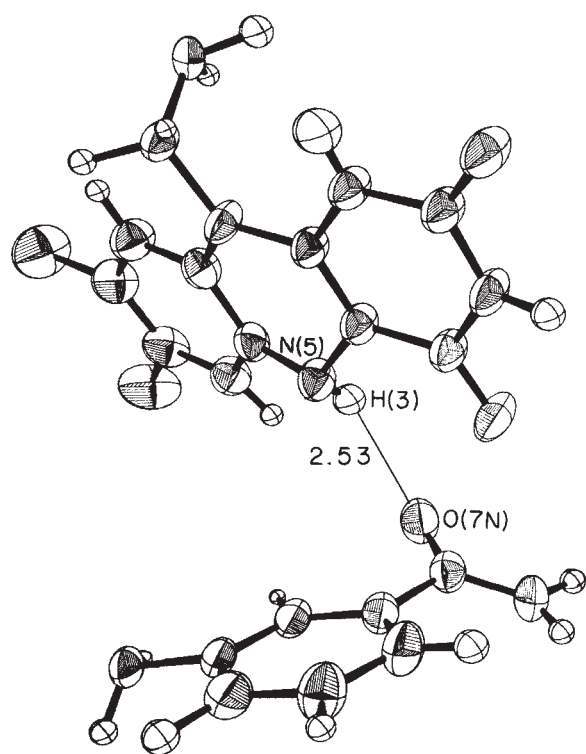


Fig. 6 A perspective drawing illustrating the hydrogen bonding association of the flavin and nicotinamide residues of neighbouring molecules in the structure of $\text{H}_2\text{Fl}_{\text{red}}\text{-C}_3\text{-Nic}^+$. Atoms are shown as thermal ellipsoids. The hydrogen bond is represented by a thin line accompanied by its length ($\text{\AA}$).

Self-consistent field molecular orbital calculations incorporating the PRDDO (partial retention of diatomic differential overlap) approximation indicate that the barrier for ring inversion in 1,5-dihydrolumiflavin has a surprisingly low value of nearly 4 kcal mol^{-1} (Lindner, D. L., Branchaud, B., Dixon, D. A., and Lipscomb, W. N., to be submitted). Nuclear magnetic resonance (NMR) measurements on several reduced flavin derivatives yield a larger but still relatively low value of 10 kcal mol^{-1} for this barrier²⁹. Therefore the observation that there is considerable flavin/flavin stacking in the crystal structure of $\text{H}_2\text{Fl}_{\text{red}}\text{-C}_3\text{-Nic}^+$ but very little ring overlap in the structures of other reduced flavins^{21,24} suggests that the small flavin bending angle in $\text{H}_2\text{Fl}_{\text{red}}\text{-C}_3\text{-Nic}^+$ is due to the influence of stacking interactions.

X-ray studies of flavodoxin from *Clostridium MP* have shown, as expected, that the flavin moiety of the bound FMN cofactor is planar in both the oxidised^{30,31} and the semiquinone^{31,32} forms of the enzyme. The flavin residue is also nearly planar in reduced *Clostridial* flavodoxin; its bending angle is estimated to be 8.6° (ref. 31). This is corroborated by the essential identity of the NMR spectra of oxidised and reduced *Clostridial* flavodoxins³³.

It has been suggested that the constraint of a protein bound reduced flavin to the planar configuration enhances its reducing power by modifying its redox potential^{29,31,34}. The nearly planar conformation of the flavin moiety in $\text{H}_2\text{Fl}_{\text{red}}\text{-C}_3\text{-Nic}^+$ cor-

roborates this hypothesis by demonstrating the flexibility of the flavin bending angle.

The red colour of $(\text{H}_2\text{Fl}_{\text{red}}\text{-C}_3\text{-Nic}^+)\text{NO}_3^-$ crystals, in contrast to the yellow colour of other crystalline reduced flavins^{21,24} might reasonably be attributed to either a charge-transfer complex or a spectral shift in the reduced flavin caused by its flattening²³. Charge-transfer interactions have been observed between stacked oxidised flavin molecules in the crystalline complex lumiflavin—2,6-diamino-9-ethyladenine³⁵ but have not been previously observed between reduced flavin molecules. The most likely site for such an interaction in the structure of $\text{H}_2\text{Fl}_{\text{red}}\text{-C}_3\text{-Nic}^+$ is within the stacks of reduced flavins. However, the fact that all interatomic contacts between reduced flavin residues are greater than van der Waals distances indicates that any charge transfer forces present would be weak at best. Likewise, the relatively small changes in the visible absorption spectrum of the nearly planar flavin molecule of reduced flavodoxin from *Clostridium MP*, in comparison with that of free reduced FMN³⁶, do not account for the red colour of crystals of $(\text{H}_2\text{Fl}_{\text{red}}\text{-C}_3\text{-Nic}^+)\text{NO}_3^-$. Hence the origin of this colour remains obscure.

Our study provides no evidence supporting the face-to-face charge-transfer complex model of flavin/nicotinamide interactions postulated on the basis of solution studies²⁻⁵. The absence of such associations in the structure of $\text{H}_2\text{Fl}_{\text{red}}\text{-C}_3\text{-Nic}^+$ corroborates the observation that these interactions are relatively weak.⁵ This is in agreement with the assertion that the characteristics of charge-transfer complexes are highly dependent on the relative orientations of the donor and the acceptor and on the contribution of other weak binding forces to the total potential energy of the complex³⁷. Thus it seems likely that the formation of any face-to-face flavin/nicotinamide complex in aqueous solutions would require stabilisation by hydrophobic forces similar to those promoting the stacking of nucleic acid bases in aqueous solutions³⁸.

We thank W. N. Lipscomb for providing the results of the molecular orbital calculations on flavins before publication. This work was supported, in part, by grants from the US NIH (GM 11040) and the MRL program of the NSF (DMR76-00678).

Received 28 March; accepted 27 June 1977.

- ¹ Massey, V. & Ghisla, S. *Ann. N.Y. Acad. Sci.* **227**, 446-465 (1974).
- ² Proffitt, R. T., Ingraham, L. L. & Blankenhorn, G. *Biochem. biophys. Acta* **362**, 534-548 (1974).
- ³ Sakurai, T. & Hosoya, H. *Biochem. biophys. Acta* **112**, 459-468 (1966).
- ⁴ Porter, D. J. T., Blankenhorn, G. & Ingraham, L. L. *Biochem. biophys. Res. Commun.* **52**, 447-452 (1973).
- ⁵ Blankenhorn, G. *Eur. J. Biochem.* **50**, 351-356 (1975); **67**, 67-80 (1976); *Biochemistry* **14**, 3172-3176 (1975); *Flavins and Flavoproteins*, Vol. V (ed. Singer, T. P.), 261-267 (Elsevier, Amsterdam, 1976).
- ⁶ Bruce, T. C., Main, L., Smith, S. & Bruce, P. Y. *J. Am. chem. Soc.* **93**, 7325-7328 (1971).
- ⁷ Gumbley, S. J. & Main, L. *Tetrahedron Lett.* 3209-3212 (1976).
- ⁸ Steffens, J. J. & Chipman, D. M. *J. Am. chem. Soc.* **93**, 6694-6696 (1971).
- ⁹ Bruce, T. C. *Prog. bioorg. Chem.* **4**, 1-87 (1976).
- ¹⁰ Brüstlein, M. & Bruce, T. C. *J. Am. chem. Soc.* **94**, 6548-6549 (1972).
- ¹¹ Fisher, J. & Walsh, C. J. *J. Am. chem. Soc.* **96**, 4345-4346 (1974).
- ¹² Porter, D. J. T. & Voet, D. submitted to *Acta crystallogr.*
- ¹³ Main, P., Woolfson, M. M. & Germain, G. *MULTAN. A Computer Program for the Automatic Solution of Crystal Structures* (Department of Physics, University of York, York, UK, 1971).
- ¹⁴ Voet, D. & Rich, A. *Prog. Nucl. Acid Res. molec. Biol.* **10**, 183-265 (1970).
- ¹⁵ Wang, M. & Fritchie, C. J. *Acta crystallogr.* **B29**, 2040-2045 (1973).
- ¹⁶ Voet, D. *J. Am. chem. Soc.* **95**, 3763-3770 (1973).
- ¹⁷ Johnson, P. L., Maier, C. A. & Paul, I. C. *J. Am. chem. Soc.* **95**, 5370-5377 (1973).
- ¹⁸ Freeman, G. R. & Bugg, C. E. *Acta crystallogr.* **B30**, 431-443 (1974).
- ¹⁹ Herriott, J. R., Cammerman, A. & Deaneau, D. R. *J. Am. chem. Soc.* **96**, 1585-1589 (1974).
- ²⁰ Singh, C. *Acta crystallogr.* **19**, 861-864 (1965).
- ²¹ Kierkegaard, P. et al. in *Flavins and Flavoproteins*, Vol. III (ed. Kamen, H.) 1-22 (University Park, Baltimore, 1971).
- ²² Wright, W. B. & King, G. S. D. *Acta crystallogr.* **7**, 283-288 (1954).
- ²³ Dudley, K. H., Ehrenberg, A., Hemmerich, P. & Müller, F. *Helv. Chim. Acta* **47**, 1354-1383 (1964).
- ²⁴ Leijonmarch, M. & Werner, P.-E. *Acta chem. scand.* **25**, 2273-2290 (1971).
- ²⁵ Wang, M. & Fritchie, C. L. Jr *Acta crystallogr.* **B29**, 2040-2045 (1973).
- ²⁶ Porter, D. J. T., Voet, J. G. & Bright, H. J. *J. Biol. Chem.* **248**, 4400-4416 (1973).
- ²⁷ Anderson, B. M. & Kaplan, N. O. *J. Biol. Chem.* **234**, 1226-1232 (1959).
- ²⁸ von Glehn, M. & Norrestam, R. *Acta chem. scand.* **26**, 1490-1492 (1972).
- ²⁹ Tauscher, L., Ghisla, S. & Hemmerich, P. *Helv. chim. Acta* **56**, 630-644 (1973).
- ³⁰ Burnett, R. M. et al. *J. Biol. Chem.* **249**, 4383-4392 (1974).
- ³¹ Ludwig, M. L. et al. in *Flavins and Flavoproteins*, Vol. V (ed. Singer, T. P.), 393-404 (Elsevier, Amsterdam, 1976).
- ³² Anderson, R. D. et al. *Proc. natn. Acad. Sci. U.S.A.* **69**, 3189-3191 (1972).
- ³³ James, T. L., Ludwig, M. L. & Cohn, M. *Proc. natn. Acad. Sci. U.S.A.* **70**, 3293-3295 (1973).
- ³⁴ Ludwig, M. L. et al. *Cold Spring Harb. Symp. quant. Biol.* **36**, 369-380 (1971).
- ³⁵ Scarbrough, F. E., Shieh, H.-S. & Voet, D. *Proc. natn. Acad. Sci. U.S.A.* **73**, 3807-3811 (1976); *Acta crystallogr.* **B33**, (in the press).
- ³⁶ Ghisla, S., Massey, V., Lhoste, J. M. & Mayhew, S. G. *Biochemistry* **13**, 589-597 (1974).
- ³⁷ Shikim, M. A. *Physico-Chemical Properties of Nucleic Acids* **1**, (ed. Duchesne, J.), 67-98 (Academic, New York, 1973).
- ³⁸ Ts'o, P. O. P. *Fine Structure of Proteins and Nucleic Acids* (eds Fasman, G. D. & Timasheff, S. N.) 49-190 (Marcel Dekker, New York, 1970).

Possible regulatory function of acetylcholine receptor in maintenance of retinotectal synapses

John A. Freeman

Vanderbilt University Medical School, Nashville, Tennessee 37232 and National Institute for Medical Research, London NW7, UK

α -Neurotoxins bind to cholinergic receptor, block transmission, and induce sprouting of retinal terminals in the toad tectum. New connections retain an orderliness that suggests a selective affinity between presynaptic terminals. The results suggest that postsynaptic cells exert a control, associated with receptors, on the growth of presynaptic terminals and on the maintenance of their synaptic connections.

THE effects of the presynaptic element of a synapse on the physiological and metabolic properties of its postsynaptic component have been extensively studied in several systems. But little is known about the influences which the postsynaptic element of a synapse might exert on the selection, maintenance, or metabolic control of its presynaptic component. Such information might provide important insights into the molecular mechanisms underlying growth, repair, and the response to injury in the central nervous system (CNS). The amphibian retinotectal system provides a useful model. The retina projects in an orderly visuotopic map on to the tectum, where the terminals from different classes of ganglion cells form synapses in separate layers^{1,2}. Moreover, the optic nerve will regenerate³, and in doing so re-establish a nearly identical pattern of connections^{4,5}.

Functional connections require a matching between neurotransmitter and postsynaptic receptor protein. Evidence has recently been obtained that acetylcholine (ACh) is an excitatory neurotransmitter in the amphibian optic tectum, and might be utilised by at least two different classes (I and IV) (ref. 1) of retinal ganglion cells. For example, (1) the retinotectal synaptic layers contain high levels of ACh, choline acetyltransferase, and ACh-esterase⁶; (2) tectal cells are excited by iontophoretically-applied ACh and synaptic responses are blocked by nicotinic antagonists⁷; (3) the reversal potentials for both iontophoretically applied ACh and the native transmitter are the same, indicating similar permeability mechanisms (manuscript in preparation); and (4) optic nerve terminal-associated synaptosomes possess a high affinity uptake system for choline, and also bind snake venom α -neurotoxins (J. A. F. and R. Oswald, in preparation). In addition, α -bungarotoxin (α -BTX) and related polypeptide α -neurotoxins, which selectively bind to nicotinic acetylcholine receptor protein (ACh-R) at neuromuscular junction^{8,9}, also bind to synapses in the tectal neuropil^{10,11}, and produce a long-lasting abolition of retinotectal transmission^{7,10}. Light autoradiographs using tritiated α -BTX reveal a distinct trilaminar distribution of receptor sites^{10,11} corresponding to the distribution of retinal terminals.

These experiments were designed to determine whether receptor-mediated changes in transmission affecting the postsynaptic cell influence the growth of presynaptic terminals. I report here that dynamic changes in the patterns of retinotectal connections occur after application of α -neurotoxins to tectal neurones in the South American toad *Bufo marinus*. The results, obtained by the use of a new mapping technique, suggest that the maintenance of synapses requires functional interaction between transmitter and receptor.

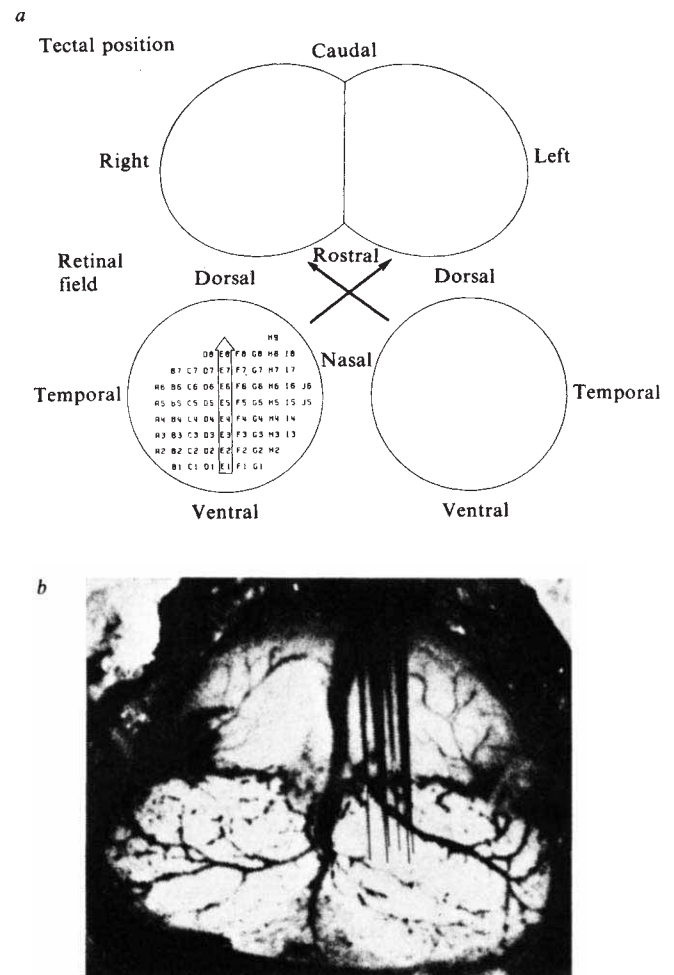


Fig. 1 Retinotectal mapping technique. Animals were immobilised either by spinal epidural anaesthesia (0.3 ml of 0.5% lidocaine in 2% sucrose) or by cooling the trunk and limbs in ice, and the tecta exposed, using a local anaesthetic. Spontaneous eye movements were eliminated by the use of a small ring held against the sclera. The optical axis of the eye, determined ophthalmoscopically, was centred on a Tektronix 611 CRT screen on which were presented electronically-generated patterns whose shape, position and brightness were controlled by a PDP-12 computer. The size and position of the equivalent retina image was computed from the translation-refraction matrix of the reduced eye. A standardised grid of retinal loci (a) was activated covering 140° of solid visual angle, and for each retinal locus the corresponding area of representation in the contralateral tectum was determined by measuring the laminar field potentials with an array of six microelectrodes (b), consisting of glass micropipettes filled with toad Ringer's, whose tips (bevelled for easier penetration¹²) were positioned 100 μ m apart in a 2 $\times$ 3 grid. Computer-averaged field potentials were combined with previously determined extracellular resistivities to compute the three-dimensional current source density (CSD), thereby providing a clear separation of pre- and postsynaptic responses, facilitating a direct comparison of maps to be made between different animals, or in the same animal mapped at different times.

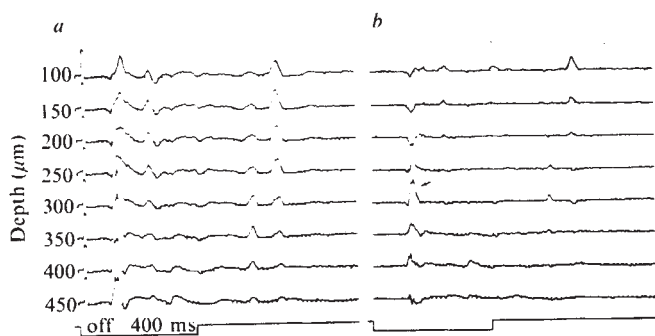


Fig. 2 Laminar field potentials (*a*) and corresponding CSDs (*b*) generated in optic tectum at indicated depths beneath the surface by the turning off of a 2° spot of light. 'Off' fibres produce a prominent sink at a depth of 300 μm, the postsynaptic component of which (arrow) is associated with a set of superficial (100–200 μm) and deep (450 μm) sources drawn from radially oriented tectal dendrites. The more superficial (100–150 μm) longer latency class II fibres are also clearly localised in (*b*). Calibration pulses at beginning of field potential records: 500 μV, negativity upwards. Same amplitude on CSD records corresponds to 10 mA cm⁻³, sink upwards.

Mapping retinotectal projections

To detect subtle changes that might occur in the positions of retinal terminals, a new mapping technique was devised, in which the retina was stimulated visually at regularly spaced loci (Fig. 1). The actual tectal location of the retinal terminals thus activated was determined by searching the tectum with an array of six microelectrodes and computing the extracellular current source density (CSD) (refs 13–15). The CSD, obtained by computing the divergence of the field potential weighted by the extracellular conductivity, represents the net transmembrane flux in a specified volume of tissue¹⁴. For measurements obtained at an interelectrode spacing of *N*, the CSD localises activity originating in an interval of *N*/2 (or 50 μm in the present study)¹⁵.

It has recently been shown¹⁶ that an extracellular microelectrode selectively records the activity of retinal terminals (which branch extensively, thereby providing a greatly increased extracellular current) rather than of fibres of passage, although it records postsynaptic activity as well. An additional advantage of the CSD technique is that it readily distinguishes between pre- and postsynaptic activity^{2,13,15}.

The following results deal primarily with class IV retinal terminals ('off' fibres), which respond selectively to a sudden decrease of illumination of their receptive fields. These terminals, which synapse on dendrites of tectal neurones whose somas lie in the central grey strata¹⁷, occupy a compact lamina 300 μm below the tectal surface. Their location is shown in the CSD records of Fig. 2*b*.

Separation of visually evoked potentials into pre- and postsynaptic components by CSD analysis is shown more clearly in Fig. 3, which also demonstrates the nicotinic cholinergic nature of class IV retinotectal synapses. In Fig. 3*a*, a 10⁻⁵ M solution of *d*-tubocurarine was applied to the tectal surface. At time 'zero' (control), the CSD waveform computed from responses to visual stimuli consists of two components: an early presynaptic sink, followed by a postsynaptic sink produced by dendritic depolarisation. This second component is gradually abolished over the next 15 min, and returns to near control values after 60 min. Figure 3*b* shows the CSD responses obtained at the same location in response to electrical stimulation of the optic nerve, after application to the tectal surface of a 10⁻⁹ M solution of α -BTX. Initially (time zero) the early presynaptic sink is followed by a prominent postsynaptic sink, which, however, is irreversibly abolished after 180 min. The toxin produced no detectable change in the presynaptic response.

Experiments were performed in eight animals to determine the normal sequence of regeneration of retinal fibres after a lesion of the optic nerve. The projections of the intact optic nerve were first determined by CSD mapping. One optic nerve was then gently crushed at a distance of approximately 2.5 mm from the optic chiasm, and each animal, maintained at 20–24 °C, was remapped at various times thereafter. The earliest evidence of re-entry of regenerating fibres into the tectum occurred at 10–14 d. The earliest patterns of presynaptic terminals were highly variable with little if any organisation. Over the ensuing 2–3 weeks considerable organisation occurred, and by 6–8 weeks after the lesion the pattern of retinal projections differed very little from the original pattern.

Effect of toxin

application on retinotectal projections

α -BTX was applied to a discrete region of tectum by placing a small square of Millipore filter soaked in toxin (5 × 10⁻⁹ M)

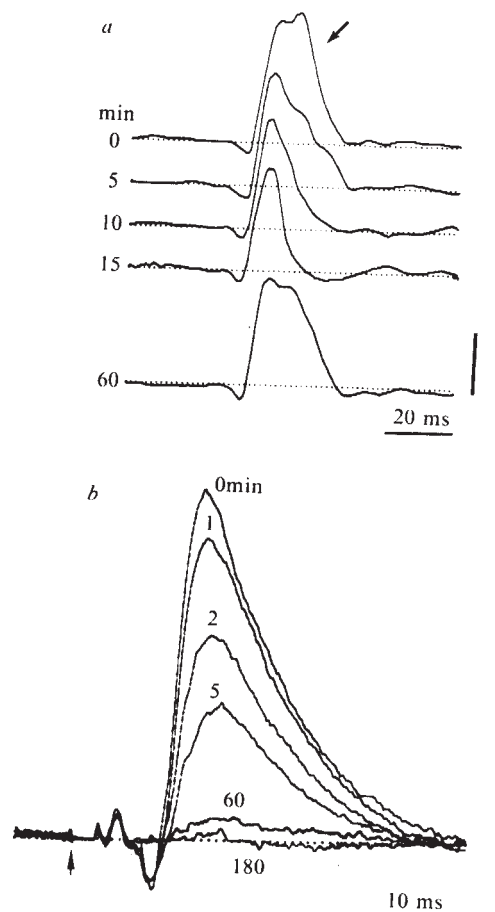


Fig. 3 Abolition of postsynaptic response to 'off' fibres by nicotinic antagonists. The lyophilised venom of *Bungarus multicinctus* was purified by chromatography on SP-Sephadex (C-25). The peak corresponding to α -BTX was collected and shown to be homogeneous on polyacrylamide gel electrophoresis using the technique of Reisfeld *et al.*¹⁸. An *in vivo* assay of toxicity was performed on toad sartorius muscle, using intracellular recording. A dose of 1 × 10⁻⁹ M caused complete loss of synaptic transmission within 30 min, with no discernable loss of presynaptic activity recorded simultaneously with a second extracellular microelectrode. In (*a*), a 10⁻⁵ M solution of *d*-tubocurarine caused reversible block of transmission in which the postsynaptic component (arrow) is gradually abolished in 15 min, but returns to near control value after 60 min. CSD records computed at 300 μm depth, following turning off of a 3° light spot at beginning of each record. In (*b*), 1 × 10⁻⁹ M α -BTX caused irreversible block of postsynaptic response to electric stimulation of optic nerve (arrow). Stimulus artefact was electronically suppressed. Average of 50 responses in (*a*), and 5 responses in (*b*). Calibration: 5 mA cm⁻³ in (*b*), sink upwards.

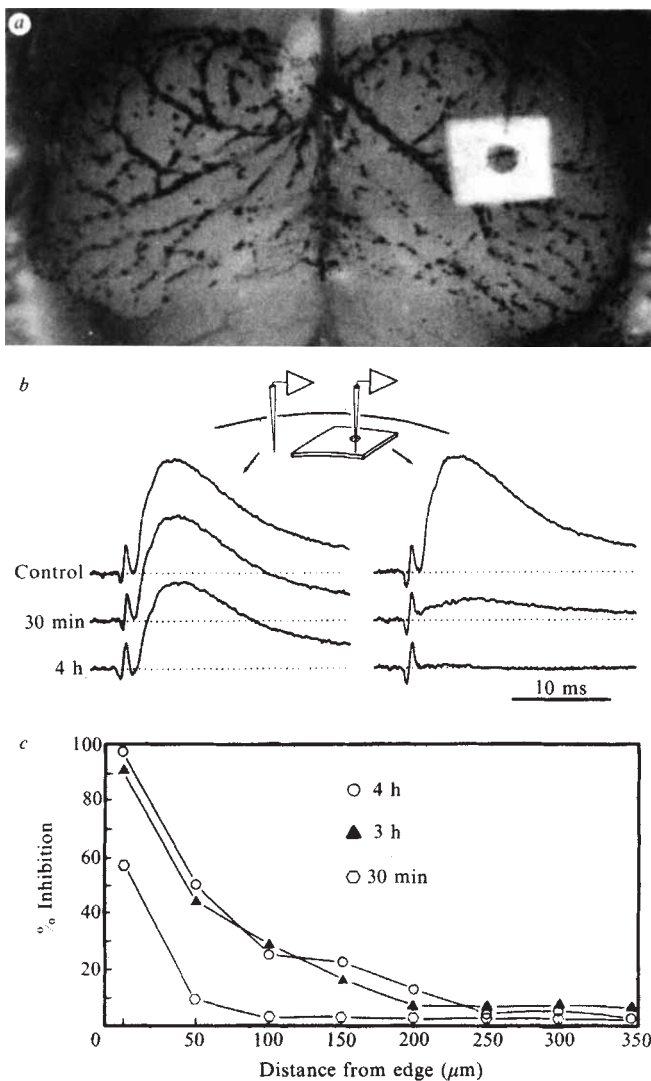


Fig. 4 Application of α -BTX to discrete region of tectum. A small square (approximately $700 \times 700 \mu\text{m}$) of α -BTX-soaked ($5 \times 10^{-9} \text{ M}$) Millipore filter was placed on the tectal surface (a). Two microelectrodes, one (dark shadow) inserted through a central hole, and the other inserted at various distances from the edge of the filter, were used to compute current source densities from responses to class IV fibres activated by shocking optic nerve. Postsynaptic responses were blocked immediately underneath filter (b, right side) but were relatively unaffected $250 \mu\text{m}$ from edge of filter (b, left side) over a 4-h period. (c) Inhibition (relative to control value before toxin application) of postsynaptic responses at indicated times and positions with respect to one edge of filter. Lack of synaptic blockade beginning approximately $250 \mu\text{m}$ outside of toxin-treated zone at 4 h (open circles) shows that little toxin diffused away from region of application.

on the tectal surface. To determine the extent of spread of toxin applied in this manner, a small square of filter was prepared with a hole cut in its centre, through which a microelectrode was lowered as shown in Fig. 4a. In Fig. 4b, the postsynaptic responses to optic nerve stimulation were progressively abolished underneath the filter over a period of 4 h, but were not significantly reduced outside of a region beginning approximately $250 \mu\text{m}$ from a side, presumably due to low diffusional mobility of toxin coupled with a high affinity of toxin-binding sites located under the region of application. The spatial and temporal spread of postsynaptic blockade produced by locally applied α -BTX is shown in Fig. 4c.

In seven animals, a small square of Millipore filter soaked in $5 \times 10^{-9} \text{ M}$ α -BTX was applied to the tectal surface two weeks after lesioning the contralateral optic nerve, when the re-

generating retinal fibres were beginning to re-enter the tectum. The filter was left in place for 4–5 h, its position photographed for later comparison, and then removed, and the animals were subsequently remapped at various times thereafter. Figure 5 shows the results of one such experiment. The map illustrated in Fig. 5b, obtained 20 d post-lesion (6 d after toxin application), shows the presence of presynaptic terminals within the toxin-treated zone, but otherwise has little organisation. By contrast, in the map obtained 14 d later (Fig. 5c), the toxin-treated zone lacked any evidence of presynaptic terminals, which instead seem to be clustered and compressed about its margins. Similar results were obtained from five other animals. On the other hand, three control animals (in which the filter was soaked in toad Ringer's rather than in α -BTX) mapped at this time, as well as another group of four animals mapped 20–30 weeks after toxin application, had an essentially normal pattern of projection with no evidence of compression.

One explanation for these effects might be that toxin killed the cells in the zone where it was applied, but that they subsequently regenerated. This seems unlikely, inasmuch as neurones remained normally responsive to other (for example, diencephalic¹⁹) inputs after toxin application, as shown by CSD analysis and single unit recording performed both during the early period (3–8 d) when toxin might be expected to produce a maximum effect, as well as at later times (2–6 weeks). A second possibility is that toxin prevented the formation of synapses. Similar experiments^{20,22} with chronic α -BTX application at the myoneuronal junction suggest that nerve-muscle synapses can in fact form when ACh-R is bound with toxin (although the possibility that they form only after bound toxin has been displaced from the receptor has not been conclusively demonstrated). A third possibility is that synapses, initially formed between regenerating terminals and α -BTX-treated tectal neurones, failed to be maintained, and that the presynaptic element continued its growth in search of a surface on which it could form an enduring synapse.

To test the effect of α -BTX on the maintenance of retino-tectal synapses, toxin was applied to the intact tectum in six animals, and the retinotectal projections were subsequently mapped at intervals of 7 d thereafter. The results from one animal are shown in Fig. 6, and are similar to those obtained from the others. One week after toxin application little if any presynaptic activity could be elicited from the toxin-treated zone (Fig. 6b) and the presynaptic terminals seemed to have shifted position and to have compressed into the borders surrounding it, displacing terminals previously located there (Fig. 6a). As shown by CSD analysis, some aberrant terminals evoked postsynaptic responses, signifying the formation of functional synapses, although the postsynaptic response amplitude was quite variable and was not easily quantitated. Maps made two weeks later (Fig. 6c) have essentially returned to normal, again suggesting that a dynamic, neuroplastic re-ordering of terminals has occurred.

These results show that the loci from which retinal terminals can be recorded change in a characteristic way after application of α -BTX. To account for these results, possible causes for the apparent sprouting, displacement, and subsequent reordering of retinal terminals should be considered.

Sprouting of retinal terminals

Several possibilities could account for the apparent shift in position of retinal terminals following the application of α -BTX shown in Figs 5 and 6, including (1), migration of postsynaptic cells²³ and their inputs; (2), unmasking of 'silent' terminals^{24,25} normally present and (3), active growth (that is, sprouting) of terminals. The first possibility is unlikely, since cells in toxin-treated regions which normally receive convergent inputs from both retinal and non-retinal (for example, diencephalic) afferents continued to respond to the latter, and were thus not likely to have migrated. The second possibility is also unlikely, since any 'silent' retinal terminals would have de-

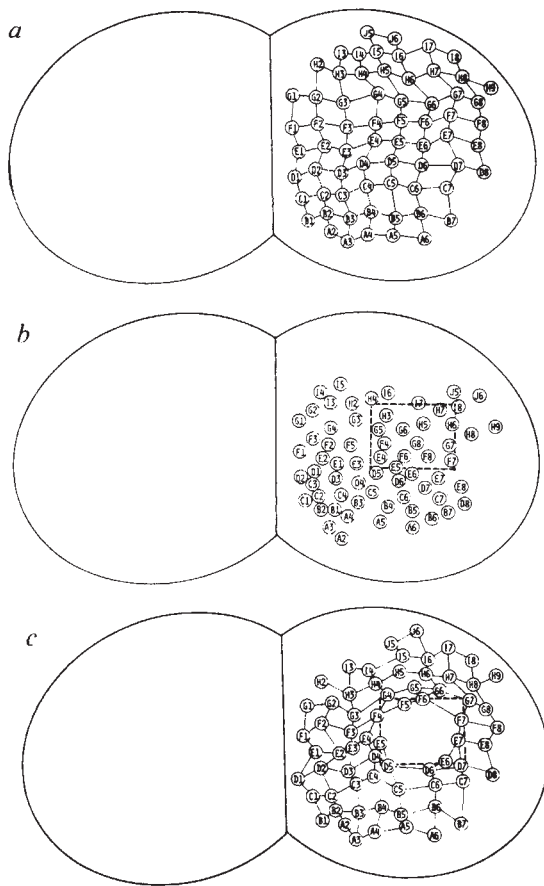


Fig. 5 Effect of localised application of α -BTX on pattern of regenerating retinal terminals. Control map shown in (a), representing positions (computed by CSD analysis) of class IV retinal terminals activated by stimulation of contralateral retinal loci shown in Fig. 1(a). Corresponding pattern of regenerating terminals shown in (b), 20 d after lesion of contralateral optic nerve, and 6 d after application of α -BTX to region indicated by dashed lines. Note the presence of terminals within the toxin-treated zone, and the general lack of organisation of the regenerating terminals. Considerable order has been restored in (c), made two weeks later. At this time, however, the toxin-treated zone is almost completely devoid of terminals, which appear clustered about its borders.

generated following optic nerve lesions. It is likely, therefore, that the results represent active growth of retinal terminals in response to toxin application, which as shown in the acute experiments of Figs 3b and 4b blocked synaptic transmission. Chronic block of neuromuscular transmission with tetanus²⁶ or with botulinum²⁷ toxin has been shown to result in sprouting of presynaptic terminals, which is suppressed by implantation of an extra nerve²⁸. Together, these results suggest that the postsynaptic element of a synapse might exert a trophic influence, dependent on the status of functional transmission, on the presynaptic element.

Similarly, the absence of detectable presynaptic activity in Figs 5c and 6b could also be accounted for by terminals growing away from the toxin-treated zones. On the other hand, some of the original terminals may have remained in the zone, but failed to produce sufficient extracellular current to be detected by the CSD technique if either the number or surface area was substantially decreased, or if chronically applied toxin interfered with the generation of action potentials (for example by binding to presynaptic ACh-R (ref. 29) or some other membrane component and altering membrane permeabilities or molecular structure). Chronic application of α -BTX for 10 d did not, however, produce any detectable decrement in action potentials in either optic nerve or sciatic nerve. Alternatively, terminals

may have degenerated, due to some primary effect of α -BTX, trace amounts of β -BTX (ref. 30) (not detectable by chromatography or gel electrophoresis), or a secondary loss of some required trophic factor. A recent³¹ electron microscopic examination of rat neuromuscular junction chronically treated with α -BTX showed no evidence of degeneration of nerve terminals, and I found no evidence of terminal degeneration in preliminary light microscopic examinations of toxin-treated regions, but detailed EM examination has not yet been done.

Displacement of retinal terminals

Maps made one week after toxin application (Fig. 6b) reveal an apparent displacement of terminals normally occupying the borders of the toxin-treated zone. Some terminals in these border regions seemed to have been displaced by as much as 250 μ m whereas terminals normally located within the toxin-treated zone seemed to have been displaced by as much as 600–700 μ m. Since the accuracy with which the eye's optical

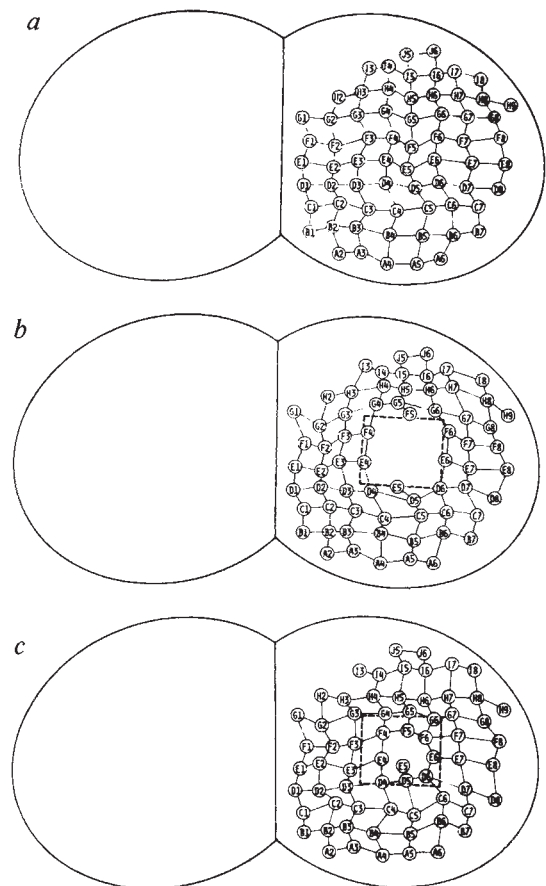


Fig. 6 Plastic changes in positions of class IV terminals following localised application of α -BTX to normally innervated tectum. Control map shown in (a). In (b), obtained one week later after applying square of Millipore filter soaked in α -BTX to region indicated by dashed lines, terminals previously located there (for example, E4-E6, F4-F6, G5) have shifted position and compressed into regions near borders, displacing terminals previously located there. In (c), obtained two weeks later, terminals have essentially returned to their original positions. Quantitative measurements of the amount of compression and order in the projections of displaced terminals in (b) were made by computing the mean distance between each recorded terminal site and its eight nearest neighbours, as well as its eight 'appropriate' neighbours. The results showed that a displaced terminal had a higher probability of retaining the same relative relationships with its displaced neighbours as with its original neighbours than any other relationship. This suggests that some selective interaction exists between terminals of neighbouring retinal ganglion cells.

axis can be precisely realigned is limited, the absolute displacement is less certain than is the relative displacement. This may represent a change in dendritic morphology of tectal neurones, or a true shift in the alignment of pre- and postsynaptic elements. An effort to differentiate between these two possibilities was made by mapping the lateral extent of antidromic field potentials generated by dendrites^{16,32} in response to focal microstimulation of tectal neuronal cell bodies in the central grey strata, by a stimulating microelectrode. The mean diameter of tectal dendritic fields estimated in this way was $110 \pm 20 \mu\text{m}$. No significant variation of dendritic field size was found within or surrounding the toxin-treated zone. This suggests that the displacement in the position of terminals in the border zones represents a true change in alignment of pre- and postsynaptic elements. As seen in Figs 5c and 6b, displaced terminals tended to retain their original orientation with respect to each other rather than to distribute randomly. The retention of a high degree of order between both invading as well as displaced terminals suggests the existence of a selective interaction between neighbouring terminals.

Reordering of retinal terminals

If the preceding interpretations are correct, both the original displacement and the subsequent reordering of retinal terminals seen in Fig. 6c represent sprouting. One would expect the sprouting terminals to ramify in the vicinity of the receptor-blocked cells, and to form contacts there. The apparent failure for this to occur implies that sites capable of receiving synapses did not exist on the receptor-blocked cells, and that the sprouting terminals did not induce their formation. In muscle, synapses occur at sites of high receptor density³³⁻³⁵, although membrane components other than the receptor are probably required for synapse formation³⁶ and recognition³⁷, as well. The proliferation of extra-junctional ACh-R that occurs in muscle after transmission block with α -BTX is significantly less than that which occurs following denervation³⁸, and is primarily related to disuse³⁸⁻⁴⁰. Since, in the present experiments, tectal cells treated with α -BTX were still driven by other inputs, there may have been relatively little stimulus of new receptor synthesis⁴¹. If synapse formation in CNS neurones requires a region of high receptor density, these results suggest that the proliferation of new ACh-R on receptor-blocked cells is slow with respect to the dynamics of the sprouting mechanism. Although the latter seems likely to involve a change in the sequence of gene activation in the ganglion cell nucleus, the expression of which will be delayed by the time required to transport materials between the cell body and its axonal terminals⁴², sprouting is known to occur rapidly in other systems in response to denervation⁴³.

It is possible that the stimulus for the apparent growth of terminals back into the toxin-treated zone occurred as a result of factors independent of the cells there (for example, crowding, non-optimum matching of molecules on pre- and postsynaptic elements, functional mismatch of information carried by displaced terminals, or a spontaneous tendency to sprout). Conversely, the question arises as to whether the re-establishment of the original pattern of terminals after toxin application is associated with the appearance of unbound receptors. The latter would be brought about by loss of toxin due to dissociation and proteolysis, and by receptor turnover, in addition to any stimulated increase in synthesis. Kouvelas and Greene⁴⁴ measured a half life of 5.1 h for α -BTX-ACh-R complex in synaptosomal fractions of chick optic tectum. A half-life of 26 h was obtained in synaptosomal fractions of toad optic tectum, using similar techniques (R. Oswald and J.A.F., in preparation). Toxin-receptor dissociation in subcellular fractions may differ significantly from that in the intact membrane, due to possible alterations of the normal molecular interactions affecting receptor conformation⁴⁵. Light autoradiographs made one week after ^3H - α -BTX application in toad tectum still showed a distinct trilaminar pattern of silver

grains (J.A.F. and W. A. Lutin, in preparation) corresponding to the distribution of retinal terminals⁴⁶. This would suggest that toxin-receptor complex is more stable *in situ* than in membrane fractions, and that its removal is slow. The turnover rate of ACh-R in brain has not yet been measured, although Lajtha *et al.*⁴⁷ have shown that 94% of membrane proteins (presumably including ACh-R) labelled with ^{14}C -tyrosine had a mean half life of 10 d. The above observations suggest that the effects of α -BTX are likely to persist for several days.

If re-ordering of retinal terminals in Fig. 6c is associated with the molecular dynamics of ACh-R, how is information about the state of surface receptors communicated to the displaced terminals? Either some terminals must remain in the toxin-treated zone allowing for direct communication, or the cells there must liberate (or fail to liberate) some factor, sensed by the displaced terminals, which affects their growth. It is possible that some trophic factor, metabolically linked to the synthesis of ACh-R, is involved in the re-ordering of synapses.

If these ideas are correct then the receptor protein might have a role in determining the postsynaptic cell's own innervation. Although the acetylcholine receptor apparently does not have a critical role in the molecular recognition or initial formation of synaptic sites, it does seem to have such a role in their maintenance, and might participate in structuring the patterns of synaptic connections.

This work was supported by NIH Career Development Award EY0240 and National Eye Institute grant EY01117. I thank W. Lutin, R. Oswald, R. Brady, J. Norden, and members of the Department of Physiology, NIMR, Mill Hill, London, for their help.

Received 10 February; accepted 8 July 1977.

- Maturana, H. R., Lettvin, J. Y., McCulloch, W. S. & Pitts, W. H. *J. gen. Physiol.* **43**, suppl. 129-175 (1960).
- Chung, S. H., Bliss, T. V. P. & Keating, M. J. *Proc. R. Soc. B* **187**, 421-447 (1974).
- Sperry, R. W. *J. Neurophysiol.* **7**, 57-70 (1944).
- Gaze, R. M. *J. exp. Physiol.* **43**, 209-214 (1959).
- Maturana, H. R., Lettvin, J. Y., McCulloch, W. S. & Pitts, W. H. *Science* **130**, 1709-1710 (1959).
- Gruber, E. R. & Freeman, J. A. *M.I.T. Prog. Rep.* **116**, 266-273 (1975).
- Freeman, J. A., Lutin, W. A. & Brady, R. N. *Trans. Neurosci. Soc.* **5**, 138 (1975).
- Chang, C. C. & Lee, C. Y. *Archs int. Pharmacodyn.* **144**, 241-257 (1963).
- Changeux, J. P., Kasai, M. & Lee, C. Y. *Proc. natn. Acad. Sci. U.S.A.* **67**, 1241-1247 (1970).
- Freeman, J. A., Lutin, W. A. & Brady, R. N. *Trans. Am. Neurochem. Soc.* **6**, 143 (1975).
- Lutin, W. A., Brady, R. N., Jensen, C. F., Skene, P. & Freeman, J. A. *Trans. Neurosci. Soc.* **5**, 630 (1975).
- Brown, K. T. & Flaming, D. G. *Brain Res.* **86**, 172-180 (1975).
- Freeman, J. A. & Stone, J. in *Neurobiology of Cerebellar Evolution and Development* (ed. Llinas, R.) 421-430 (AMA Press, Chicago, 1967).
- Nicholson, C. & Freeman, J. A. *J. Neurophysiol.* **38**, 356-368 (1975).
- Freeman, J. A. & Nicholson, C. *J. Neurophysiol.* **38**, 369-382 (1975).
- George, S. A. & Marks, W. B. *Expl Neurol.* **42**, 467-482 (1974).
- Szekely, G. in *Handbook of Sensory Physiology VII/3* (ed. Jung, R.) ch. 13 (Springer, Berlin, 1975).
- Reisfeld, R. A., Lewis, U. J. & Williams, D. E. *Nature* **195**, 281-283 (1962).
- Trachtenberg, M. C. & Ingle, D. *Brain Res.* **79**, 419-430 (1974).
- Steinbach, J. H., Harris, A. J., Patrick, J., Shubert, D. & Heinemann, S. J. *gen. Physiol.* **62**, 255-270 (1973).
- Harris, A. J. in *Synaptic Transmission and Neuronal Interaction* (ed. Bennett, M. V. L.) 315-337 (Raven, New York, 1974).
- Jansen, J. K. S. & van Essen, D. C. *J. Physiol., Lond.* **250**, 651-667 (1975).
- Gaze, R. M. & Watson, W. E. in *Growth of the Nervous System* (eds Wolstenholme, G. E. W. & O'Connor, M.) 53-66 (Churchill, London, 1968).
- Marotte, L. R. & Mark, R. F. *Brain Res.* **19**, 41-62 (1970).
- Merrill, E. G. & Wall, P. D. *J. Physiol., Lond.* **226**, 825-846 (1972).
- Duchen, L. W. *J. Neurol. Sci.* **19**, 155-167 (1975).
- Duchen, L. W. *J. Neurol. Neurosurg. Psychiat.* **33**, 40-54 (1970).
- Duchen, L. W., Rogers, M., Stolk, C. & Tonge, D. A. *J. Physiol., Lond.* **241**, 1P (1975).
- Lentz, T. L., Rosenthal, J. & Mazurkiewicz, J. E. *Trans. Neurosci. Soc.* **5**, 976 (1975).
- Kelly, R. S. & Brown, F. R. *J. Neurobiol.* **5**, 135-150 (1976).
- Duchen, L. W., Heilbronn, E. & Tonge, D. A. *J. Physiol., Lond.* **250**, 26-27P (1975).
- Llinas, R., Bloedel, J. R. & Roberts, W. J. *J. Neurophysiol.* **32**, 881-891 (1969).
- Fischbach, G. D., Berg, D. K., Colson, S. A. & Frank, E. *Cold Spring Harb. Symp. quant. Biol.* **40**, 347-358 (1976).
- Fambrough, D. M. & Rash, J. E. *Dev Biol.* **26**, 55-68 (1971).
- Sytzkowski, A. J., Vogel, Z. & Nirenberg, M. W. *Proc. natn. Acad. Sci. U.S.A.* **70**, 270-274 (1973).
- Grinnell, A. D., Rheuben, M. B. & Letinsky, M. S. *Nature* **265**, 368-370 (1977).
- Appel, S. H. *Expl Neurol.* **48**, 52-74 (1975).
- Pestrouk, A., Drachman, D. B. & Griffin, J. W. *Nature* **260**, 352-353 (1976).
- Chang, C. C., Chuang, S.-T. & Huang, M. C. *J. Physiol., Lond.* **250**, 161-173 (1975).
- Lomo, T. & Westgaard, R. H. *Cold Spring Harb. Symp. quant. Biol.* **40**, 263-274 (1976).
- Roper, S., Purves, D. & McMahan, U. J. *Cold Spring Harb. Symp. quant. Biol.* **40**, 283-296 (1976).
- Grafstein, B. *Expl Neurol.* **48**, 32-51 (1975).
- Cotman, C. W. & Lynch, G. S. in *Neuronal Recognition* (ed. Barondes, S. H.) 69-108 (Chapman and Hill, London, 1976).
- Kouvelas, E. D. & Greene, L. A. *Brain Res.* **113**, 111-126 (1976).
- Changeux, J. P. *et al. Cold Spring Harb. Symp. quant. Biol.* **40**, 211-230 (1976).
- Gruber, E. R. & Freeman, J. A. *M.I.T. Prog. Rep.* **116**, 263-265 (1975).
- Lajtha, A., Latzkovits, L. & Toth, J. *Biochim. biophys. Acta* **425**, 511-520 (1976).

letters to nature

Observations of OE323(4C34.13), MO758+120, and OS210(4C28.40) with the 5-km telescope

FOUR extended radio sources identified with quasars of large redshift have been observed¹, they are important in defining the relationship between mean radio angular diameter and redshift and thus limiting the permissible range of q_0 and cosmological evolution of the radio source population. A difficulty with such analyses is that of allowing for the variation of the physical sizes of individual sources with time, particularly if there are different types of source present in the sample. The ideal would be to restrict the analysis to a single type with a comparatively small range of the relevant properties. We have, therefore, mapped three of the four sources—those accessible to the 5-km telescope—in order to study their structures with greater angular resolution and a better beam shape. We have also re-measured the optical positions of the associated quasars from the prints of the Palomar Sky Survey. Our results remove the positional discrepancy for MO758+120 (ref. 1) and confirm the association of an optical quasar with each of the radio sources. The suggestion that the optical and radio positions of MO758+120 (ref. 3) had been accidentally interchanged seems to be

incorrect, since our optical position agrees with that given by Baldwin *et al.*³ to within 0.25 arc s.

Our observing frequency was 2.7 GHz, as used by Gearhart and Pacht¹, and the beam size (FWHP) $3.7 \times 3.7 \cos \delta$ arc s; in one case, OS210, the source had previously been mapped at 5 GHz (ref. 2) with a beam size 2×4.1 arc s.

Figure 1 shows the 2.7-GHz maps in which the declination scales have been compressed so that the beams seem circular; one can then more easily detect the presence of marginally resolved components. The peak positions and flux densities of the components are given in Table 1. In the case of OS210 no central nuclear component was found, and our observations at 5.0 and 2.7 GHz may be combined with measurements of the total flux density at other frequencies to obtain a mean spectral index for the two outer components. The other two sources are found to be triples, in one of which the nucleus provides most of the flux at 2.7 GHz. To estimate the spectra of the outer components of each, an attempt was made to correct the measurements of total flux density, which are available over a wide range of frequency, by subtracting the flux density attributable to the 2.7-GHz nucleus on the assumption that its spectrum is flat. Details for the individual sources follow.

A weak central radio component lies within about 1 arc s of the optical quasar OE323, confirming the identification. The

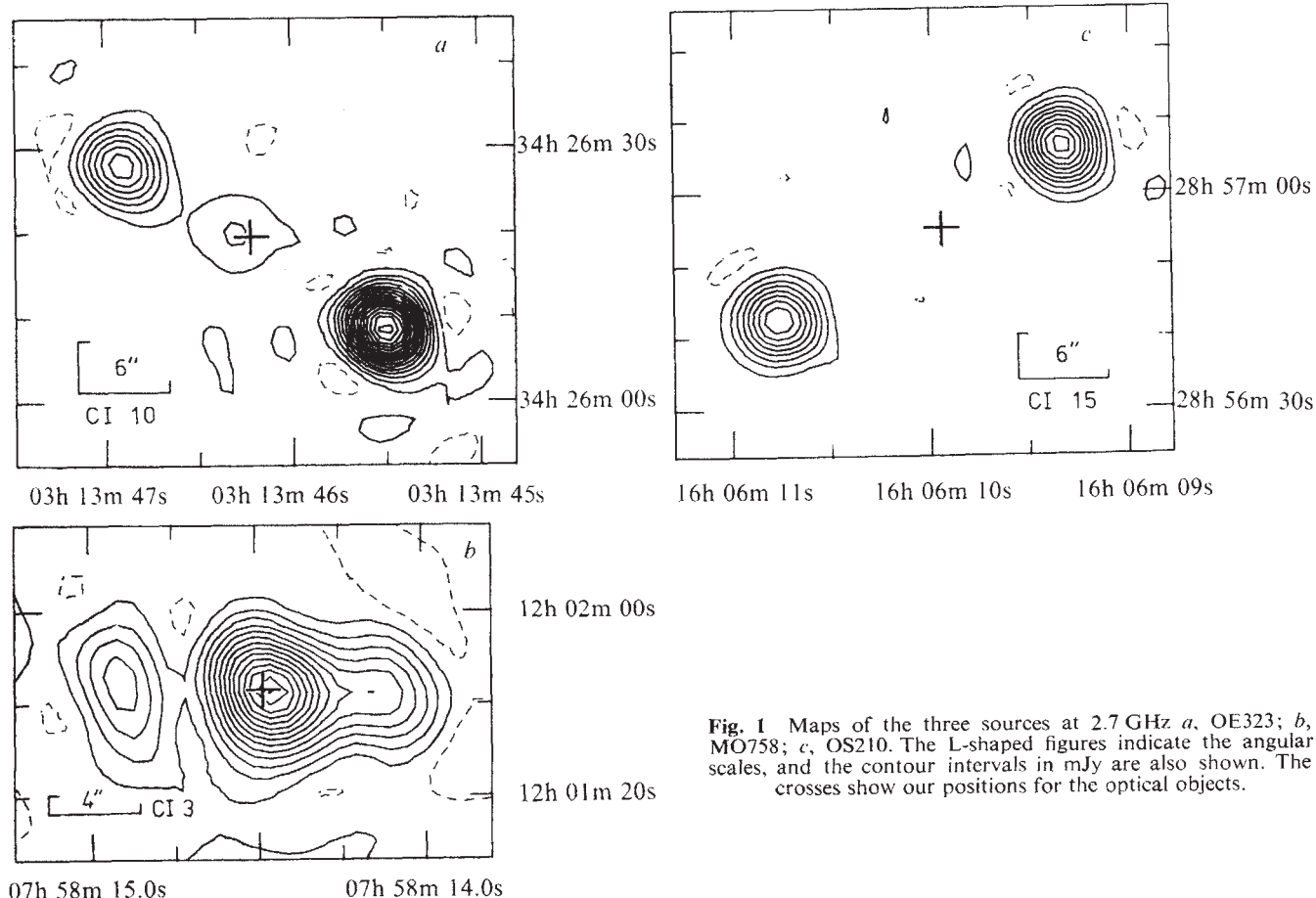


Fig. 1 Maps of the three sources at 2.7 GHz: a, OE323; b, MO758; c, OS210. The L-shaped figures indicate the angular scales, and the contour intervals in mJy are also shown. The crosses show our positions for the optical objects.

Table 1 Peak positions and flux densities

Source	Component	α (1950.0)				δ (1950.0)				$S_{2.7}(\text{mJy})$	$\pm$
		h	m	s	$\pm$ s	°	'	"	$\pm''$		
OE323	Nf	03	13	46.92	0.02	34	26	27.8	0.3	76	15
	nucleus	—	—	46.31	0.04	—	—	19.7	0.6	14	5
	optical	—	—	46.22	0.07	—	—	19.5	0.7	—	—
	Sp	—	—	45.50	0.02	—	—	08.4	0.3	192	20
MO758+120	Nf	07	58	14.92	0.03	12	01	44.2	0.6	11	5
	nucleus	—	—	14.46	0.02	—	—	42.5	0.3	39	15
	optical	—	—	14.48	0.05	—	—	43.1	0.5	—	—
	Sp	—	—	14.16	0.03	—	—	42.5	0.6	16	5
OS210	Np	16	06	09.34	0.02	28	57	06.9	0.4	166	20
	optical	—	—	09.95	0.06	—	56	55.1	0.7	—	—
	Sf	—	—	10.77	0.02	—	—	42.2	0.5	154	25

Table 2 Physical extent, total intrinsic luminosity and the relative luminosity of the nucleus to the total

Source	z	θ (arc s)	$S_{2.7}$ (mJy)	$\Omega = 0$		$\Omega = 1$		$\frac{P_N}{P_{\text{Total}}}$	α_{outer}
				D (kpc)	P_5 (10^{26} W Hz $^{-1}$ sr $^{-1}$)	D (kpc)	P_5 (10^{26} W Hz $^{-1}$ sr $^{-1}$)		
OE323	1.156	26.2	282	299	1.8	225	1.0	0.04	0.96
MO758 + 120	2.66	11.3	66	152	4.1	86	1.3	0.34	1.43
OS210	1.989	31.1	320	401	9.0	255	3.7	< 0.01	0.92

spectral index of the unresolved outer components over the range 178 to 2,695 MHz is $\alpha = 0.96 \pm 0.05$ (defined by $S \propto \nu^{-\alpha}$).

The source MO758+120 is a well-defined triple with a very powerful central component; our optical position for the quasar lies within about 0.7 arc s of the central component. Gearhart and Pacht's observation did not resolve the source, and indicated a positional difference of some 16 arc s between the radio centroid and the optical identification. The spectrum of the outer components can be derived from our 2.7-GHz results and low-frequency observations for which the correction for the nucleus is small. Over the range 38–2,695 MHz the spectrum is very steep with $\alpha = 1.43 \pm 0.02$.

OS210 is a double with unresolved components, our optical position lying within 0.8 arc s of the line joining the components. The earlier 5-GHz map shows both components to have angular sizes less than 2 arc s. Over the range 38–2,695 MHz, $\alpha = 0.94 \pm 0.02$.

In order to compare the physical characteristics of these sources, the physical extent, the total intrinsic luminosity (P) and the relative luminosity of the nucleus to the total for each were computed for the Einstein–de Sitter ($\Omega = 1$), and Milne ($\Omega = 0$) models (Table 2). To reduce the uncertainty arising from the spectrum of the nucleus, these values are extrapolated to 5 GHz, which corresponds more closely to the mean frequency of emission than to that of observation of 2.7 GHz.

It can be seen that even amongst these three sources there is a wide spread in projected diameter, luminosity and morphological type and, in attempting to distinguish between different cosmological models (including the type of source evolution involved), it will be necessary to take account of more source parameters than have been considered hitherto. The results emphasise the importance of high resolution and high sensitivity in the study of these problems.

We thank the director of the Institute of Astronomy for the use of the x-y measuring machine. M.A.C.P. thanks the SRC for a maintenance award.

M. A. C. PERRYMAN

M. RYLE

Cavendish Laboratory,
Madingley Road,
Cambridge, UK

Received 13 July; accepted 2 August 1977.

¹ Gearhart, M. R. & Pacht, E. *Nature* **266**, 819–822 (1977).

² Riley, J. M. & Pooley, G. G. *Mem. R. astr. Soc.* **80**, 105–137 (1975).

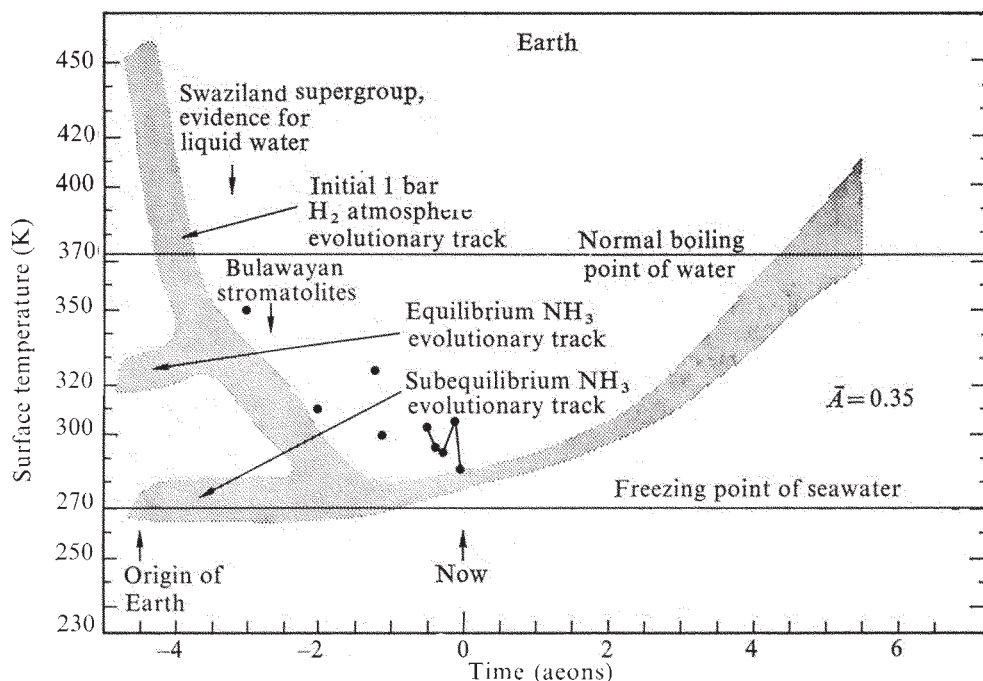
³ Baldwin, J. A. *et al. Astrophys. J.* **206**, L83–L86 (1976).

Reducing greenhouses and the temperature history of Earth and Mars

The modern theory of stellar evolution implies that the Sun has increased in brightness by several tens of per cent over geological time. Were all other global parameters held constant, this would imply that the mean temperature of the Earth was below the freezing point of seawater about 2×10^9 yr ago¹. There is, however, excellent geological and palaeontological evidence that there were extensive bodies of liquid water on the Earth between 3 and 4×10^9 yr ago. A possible solution to this puzzle, first postulated by Sagan and Mullen¹, is that the Earth's primitive atmosphere contained small quantities of NH_3 and other reducing gases which significantly enhanced the global greenhouse effect. NH_3 is especially effective in the 8–13 μm window in a CO_2 – H_2O atmosphere. It was argued that plausible changes in other global parameters, such as the infrared emissivity or the Russell–Bond albedo, would have been ineffective. The increase in solar brightness over geological time seems model-invariant even under extreme model perturbation for the solar interior¹, as has recently been reconsidered in a straightforward stellar structure scaling argument². Cosmochemical considerations point strongly to a higher abundance of reduced constituents in the primitive than in the contemporary terrestrial atmosphere; and reduced atmospheric components such as NH_3 and CH_4 are required to understand the accumulation of prebiological organic compounds necessary for the origin of life in this same period, between 3 and 4×10^9 yr ago³.

The calculations of Sagan and Mullen used a very simple square-wave opacity model, and radiative equilibrium. The model predicts the correct value of the present terrestrial global temperature (about 288 K). Calculations were carried out for three primitive atmospheres, as shown in Fig. 1: (1) a high-density hydrogen-rich primitive atmosphere where the opacity was provided primarily by pressure-induced dipole and forbidden quadrupole transitions of H_2 ; (2) a hydrogen-poor, but still reducing, primitive atmosphere in

Fig. 1 Evolutionary tracks showing the time evolution of global surface temperatures of the Earth for three different atmospheric chemistries¹. The arrows for Swaziland and Bulawayan rocks indicate two of several lines of evidence for extensive liquid water in the early history of the Earth. The points show recent measurements of surface temperatures from isotopic thermometric techniques⁴. The bolometric Russell-Bond albedo is taken as 0.35. Time is measured in aeons (1 aeon = 10^9 yr).



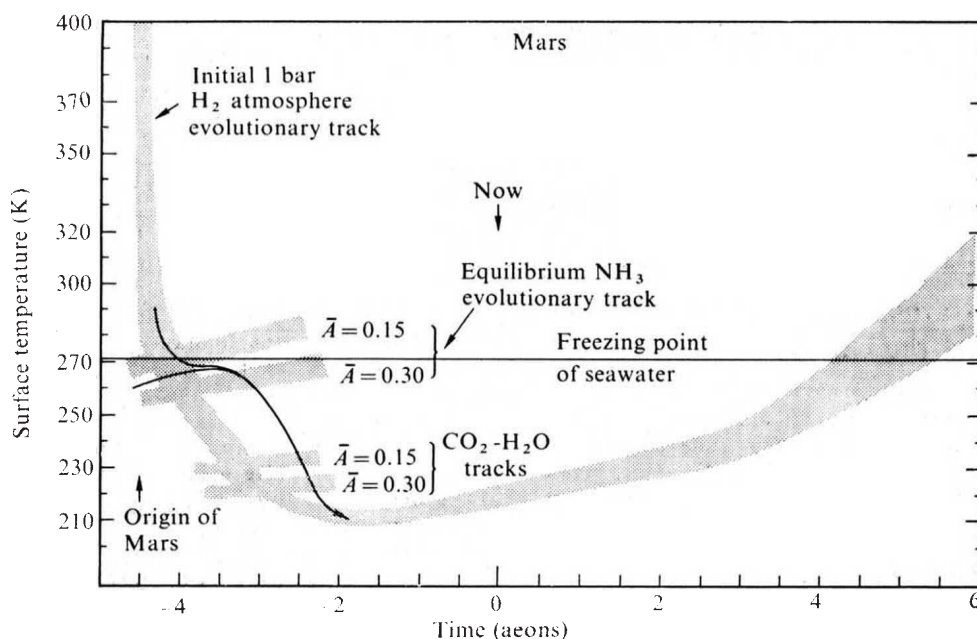
which the opacity was provided primarily by the chemical equilibrium abundance of ammonia ($\sim 10^{-4.5}$ volume mixing ratio); and (3) a hydrogen-depleted atmosphere in which the ammonia abundance was subequilibrium, due primarily to the photodissociation of NH_3 . The time dependence of these opacities was determined crudely, allowing for Jeans escape of hydrogen from the exosphere and the subsequent oxidation of reducing compounds, with a characteristic period $\sim 3 \times 10^8$ yr.

Knauth and Epstein⁴ have obtained, through $\text{D}/^{18}\text{O}$ isotopic archeothermometry, data on mean global temperatures from the present back to about 3×10^9 yr ago. These points are also shown in Fig. 1 and roughly parallel the high opacity evolutionary tracks (either 1 bar H_2 and equilibrium NH_3 , or equilibrium NH_3 alone), and provide some support for the idea of a substantial primitive greenhouse effect described in (1) above. Since the observed temperatures seem a little higher than the tracks calculated, it seems useful to point out that octapole transitions of methane, neglected in (1), have been shown by Pollack⁵ to

be significant for the greenhouse of Titan. If a primitive terrestrial atmosphere with total pressure ~ 1 bar had more than ~ 0.01 CH_4 , the CH_4 opacity would have made an important contribution to the greenhouse effect. Even if the NH_3 opacity were overestimated in (1), CH_4 and NH_3 together as minor constituents in the primitive terrestrial atmosphere seem capable of explaining in a very natural way what otherwise would be a serious discrepancy between astrophysical theory and geological observation.

The same computational protocol has been applied to the evolution of surface temperature on the planet Mars. Results are shown in Fig. 2, for two possible bolometric Russell-Bond albedos of Mars, 0.15 and 0.30, and for three conceivable early compositions: (1) a 1 bar H_2 atmosphere; (2) a 1 bar H_2 -depleted early atmosphere with NH_3 as a minor constituent; and (3) a low-pressure early atmosphere, comparable to the present martian atmosphere, in which CO_2 and small quantities of water vapour provide the principal sources of infrared opacity. Three separate Viking instruments have determined the abundances of trace

Fig. 2 Evolutionary tracks for the time dependence of surface temperature for Mars for three early compositions and two different bolometric Russell-Bond albedos.



isotopic constituents in the contemporary martian atmosphere, and have been interpreted, by a set of independent arguments, to indicate a possible higher pressure atmosphere in early martian history⁶⁻⁸. The most frequently cited estimates of total pressure are about 0.1 bar, but estimates range as high as 1 bar. Because of the low exosphere temperature, H₂ does not escape from Mars today significantly faster than it does from Earth, despite the larger gravitational acceleration of the latter.

The results indicate that if primitive Mars, like primitive Earth, had even a mildly reducing atmosphere, martian global temperatures a few times 10⁹ yr ago may have been in the vicinity of the freezing point of seawater and, in many latitudes, the mean temperatures may have been above the freezing point. This result is of possible interest for the martian sinuous channels, many of which seem to be produced by aqueous fluvial erosion and which have been crudely dated by cratering statistics techniques as having been formed 1–4 × 10⁹ yr ago⁹⁻¹³. Although the Viking imaging and microbiology experiments have so far yielded no unambiguous evidence for martian biology¹⁶⁻²⁰, the implication of this work—that in earlier martian history, temperatures and pressures may have been substantially higher in a reducing atmosphere and extensive bodies of liquid water present—certainly enhances significantly the *a priori* likelihood of the origin of life on early Mars. Of course, even if life arose, there is no guarantee that it survived subsequent inclemencies of martian history. Our results also imply, because of the increasing solar luminosity, a very clement martian epoch a few times 10⁹ yr in the future (Fig. 2), when conditions on Earth will become—due to the inexorable nature of stellar evolution—less than ideal (Fig. 1).

This research was supported by the NSF and the NASA. I thank Samuel Epstein and James Pollack for useful discussions, and George Mullen for assistance with the calculations.

CARL SAGAN

Laboratory for Planetary Studies,
Cornell University,
Ithaca, New York 14853

Received 28 March; accepted 13 July 1977.

- 1 Sagan, C. & Mullen, G. *Science* **177**, 52–56 (1972).
- 2 Newman, M. J. & Rood, R. T. *Nature* (in the press).
- 3 Sagan, C. *Origins of Life* **5**, 497–505 (1974).
- 4 Knauth, L. T. & Epstein, S. *Geochim. cosmochim. Acta* **40**, 1095–1108 (1976).
- 5 Pollack, J. B. *Icarus* **19**, 195–201 (1973).
- 6 McElroy, M. B., Yung, Y. L. & Nier, A. O. *Science* **194**, 70–72 (1976).
- 7 Nier, A. O., McElroy, M. B. & Yung, Y. L. *Science* **194**, 68–70 (1976).
- 8 Biemann, K., Owen, T., Rushneck, D., La Fleur, A. & Howarth, D. W. *Science* **194**, 76–78 (1976).
- 9 Sagan, C., Toon, O. B. & Gierasch, P. J. *Science* **181**, 1045–1049 (1973).
- 10 Milton, D. J. *J. geophys. Res.* **78**, 4009–4030 (1973).
- 11 Baker, V. R. & Milton, D. J. *Icarus* **23**, 27–41 (1974).
- 12 Milton, D. J. *Science* **183**, 654–656 (1974).
- 13 Sharp, R. P. & Malin, M. C. *Geol. Soc. Am. Bull.* **86**, 593–609 (1975).
- 14 Malin, M. C. *J. geophys. Res.* **81**, 4825–4845 (1976).
- 15 Puri, D. *Icarus* **27**, 25–49 (1976).
- 16 Mutch, T. A. *et al. Science* **193**, 791–801 (1976).
- 17 Levinthal, E. C. *et al. J. geophys. Res.* (in the press).
- 18 Klein, H. P. *et al. Science* **194**, 99–105 (1976).
- 19 Horowitz, N. H., Hobby, G. L. & Hubbard, J. S. *Science* **194**, 1321–1322 (1976).
- 20 Levin, G. V. & Straat, P. A. *Science* **194**, 1322–1328 (1976).

Stratospheric CH₄, HCl and ClO and the chlorine-ozone cycle

SINCE it was suggested¹ that chlorine may influence stratospheric ozone balance, and that halocarbons can contribute significantly to the stratospheric chlorine content²⁻⁴ much attention has been paid to the subject. We report here a new determination of atmospheric methane levels which gives a value lower than that often assumed by other workers. In model calculations this suggests that chlorine has a greater effect on the ozone balance than previously suspected.

In addition to atomic chlorine two main species of the chlorine-ozone cycle have been measured in the stratosphere—

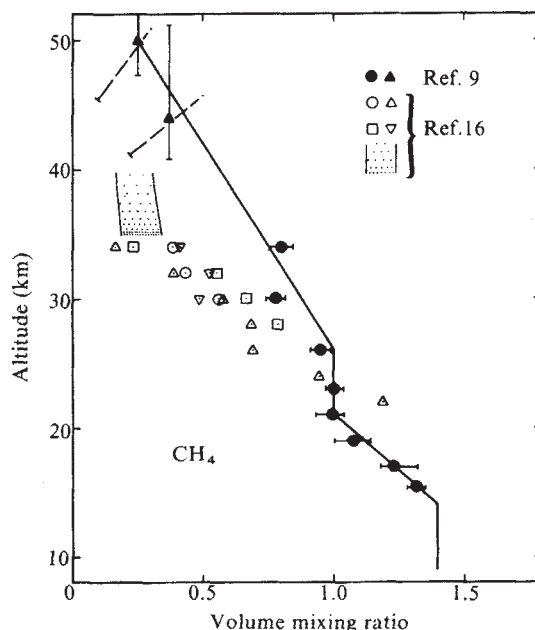
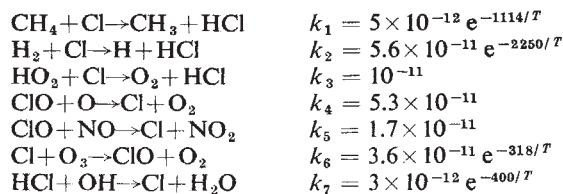


Fig. 1 Vertical distribution of the volume mixing ratio of methane in parts per million. The *in situ* sampling results⁹ are shown with the values determined¹⁶ by infrared spectrometry of the P₅ to P₈ multiplets of the 3.3-μm band. The solid curve represents the values used in model calculations⁴. The envelope above 35 km corresponds to the methane absorptions at Zenith angle, χ , smaller than 90°.

namely HCl (ref. 5) and ClO (ref. 6). The ratio of their abundances can be expressed by the relation³

$$\frac{n\text{HCl}}{n\text{ClO}} = \frac{(k_1 n\text{CH}_4 + k_2 n\text{H}_2 + k_3 n\text{HO}_2) \times (k_4 n\text{O} + k_5 n\text{NO})}{(k_6 n\text{O}_3 + k_7 n\text{OH})} \quad (1)$$

where nA represents the number density of species A and values of k expressed below in $\text{cm}^3 \text{mol}^{-1} \text{s}^{-1}$, represent the rate constants⁷ of the reactions



The $\text{H}_2\text{O}_2 + \text{Cl} \rightarrow \text{HCl} + \text{HO}_2$ reaction has been neglected due to its low rate constant⁸.

All species in very low stratospheric concentration (except HO₂ which has a minor role) intervening in relationship (1) have been measured—H₂ (ref. 9), NO (refs 10, 11), O (refs 12, 13) and OH (ref. 14). The abundance of ozone is known¹⁵ and most studies have relied on CH₄ data obtained in the stratosphere by *in situ* sampling⁹.

In the altitude range 32–36 km where data are available for computation the $n\text{HCl}/n\text{ClO}$ ratio is observed to be about 0.8 while values ranging from 4.5 to 3.5 result from model calculations⁴.

We have performed a new determination¹⁶ of CH₄ using infrared absorption spectrometry from a balloon platform to give methane mixing ratios over 25 km lower than currently used in stratospheric models. The results, shown in Fig. 1, were derived from spectra of the 3.3-μm CH₄ band obtained simultaneously with HCl absorption spectra¹⁷. They have been used in conjunction with all other measurements to evaluate the $n\text{HCl}/n\text{ClO}$ ratio from equation (1) in order to deduce the effect of these new data on the chlorine-ozone cycle. The

Table 1 Data used in the evaluation of $n\text{HCl}/n\text{ClO}$ and results

	Altitude (km)		
	32	34	36
$n\text{O}_2$ (ref. 15)	2.5×10^{12}	1.9×10^{12}	1.4×10^{12}
$n\text{CH}_4$ (ref. 16)	1.2×10^{11}	7.0×10^{10}	4.2×10^{10}
$n\text{H}_2$ (ref. 9)	2.0×10^{11}	1.4×10^{11}	1.0×10^{11}
$n\text{HO}_2$ (ref. 3)	2.2×10^7	1.9×10^7	1.5×10^7
$n\text{OH}$ (ref. 4)	1.1×10^7	1.1×10^7	1.5×10^7
$n\text{O}$ (ref. 13)	1.3×10^8	2.6×10^8	3.9×10^8
$n\text{NO}$ (ref. 11)	1.3×10^9	1.1×10^9	7.0×10^8
$T(\text{K})$ (ref. 18)	228	234	239
$n\text{HCl}$ (observed) ¹⁷	4.2×10^8	3.0×10^8	2.4×10^8
$n\text{ClO}$ (observed) ⁶	5×10^8	4×10^8	3×10^8
$n\text{HCl}/n\text{ClO}$ (equation (1))	1.2	1.2	0.8
$n\text{HCl}/n\text{ClO}$ (observed)	0.84	0.75	0.8
$n\text{HCl}/n\text{ClO}$ (model) ¹	4.5	3.9	3.5

number densities used in the computation are listed in Table 1 with the results. The computed values of the $n\text{HCl}/n\text{ClO}$ ratio seem to be in good agreement with observed values while the model calculated ratios are, especially at the lowest altitude, in marked disagreement. Part of this disagreement is due to the use in models of high methane concentrations which have already been considered as being surprisingly large by Wofsy and McElroy¹⁹. A similar opinion has been expressed by Rowland and Molina³ who have considered the possibility of lower methane concentrations leading to an enhancement by a factor of 2 or 3 of the chlorine atom chain.

Thus introduction of high methane concentrations⁹ into model calculations⁴ has led to a computed $n\text{HCl}/n\text{ClO}$ ratio, for the case considered here, 2.3 times higher than that obtained when using the lower level of methane reported here.

M. ACKERMAN

D. FRIMOUT

C. MULLER

Institut d'Aeronomie Spatiale
de Belgique,
3, avenue Circulaire,
B-1180—Brussels, Belgium

Received 16 June; accepted 12 July 1977.

- ¹ Stolarski, R. S. & Cicerone, R. J. *Can. J. Chem.* **52**, 1610–1615 (1974).
- ² Molina, M. J. & Rowland, F. S. *Nature* **249**, 810–812 (1974).
- ³ Rowland, F. S. & Molina, M. J. *Rev. Geophys. Sp. Phys.* **13**, 1–35 (1975).
- ⁴ *Halocarbons: Effects on Stratospheric Ozone* (National Academy of Sciences, Washington, DC, 1976).
- ⁵ Eyre, J. R. & Roscoe, H. K. *Nature* **266**, 243–244 (1977).
- ⁶ Anderson, J. G. *NASA Conference on the Stratosphere and Related Problems*, Logan, Utah (September, 1976).
- ⁷ Nicolet, M. *Rev. Geophys. Sp. Phys.* **13**, 593–636 (1975).
- ⁸ Hampson, R. F. & Garvin, D. *Chemical Kinetic and Photochemical Data for Modelling Atmospheric Chemistry*, Addenda of June 1975, NBS Technical Note 866.
- ⁹ Ehhalt, D. H., Heidt, L. E., Lueb, R. H. & Pollack, W. *Pageoph* **113**, 389–402 (1975).
- ¹⁰ Ackerman, M. *et al. Nature* **245**, 205–206 (1973).
- ¹¹ Ackerman, M. *J. Atmos. Sci.* **32**, 1649–1657 (1975).
- ¹² Anderson, J. G. *Geophys. Res. Lett.* **2**, 231–234 (1975).
- ¹³ Anderson, J. G. in *Halocarbons: Effects on Stratospheric Ozone* (National Academy of Sciences, Washington, DC, 1976).
- ¹⁴ Anderson, J. G. *Geophys. Res. Lett.* **3**, 165–168 (1976).
- ¹⁵ Krueger, A. J. *Pageoph* **106–108**, 1272–1280 (1973).
- ¹⁶ Ackerman, M., Frimout, D. & Muller, C. *Aeronomica Acta* **180** (1977).
- ¹⁷ Ackerman, M., Frimout, D., Girard, A., Gottignies, M. & Muller, C. *Geophys. Res. Lett.* **3**, 13–16 (1976).
- ¹⁸ *US Standard Atmosphere Suppl.* (US Government Printing Office, Washington, DC, 1966).
- ¹⁹ Wofsy, S. C. & McElroy, M. B. *J. Geophys. Res.* **78**, 2619–2624 (1973).

Defects in natural type IB diamond

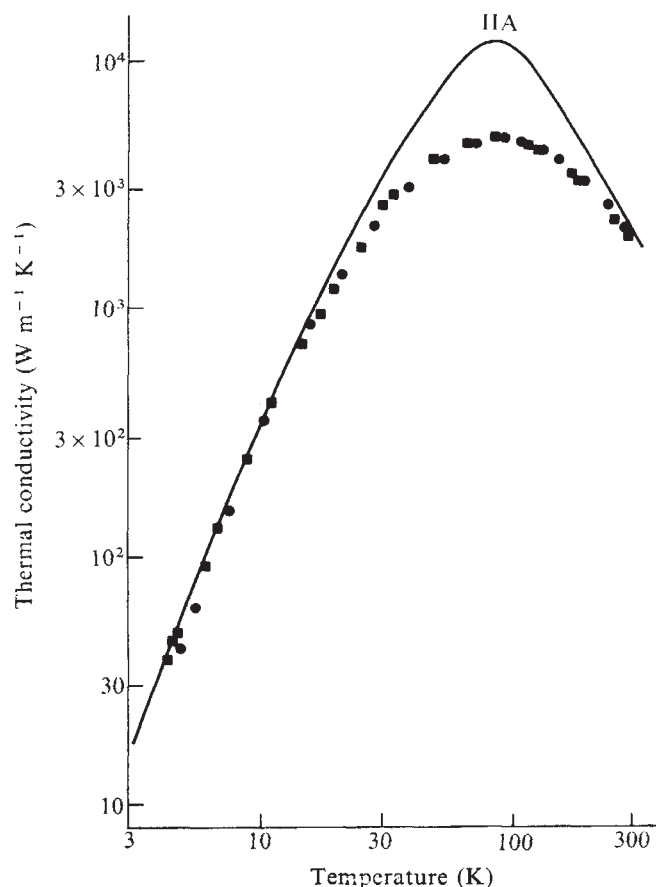
NATURAL type IB diamond has an anomalously low thermal conductivity. To investigate this we have carried out birefringence, infrared and ultraviolet absorption spectroscopy, electron spin resonance and transmission electron

microscopy (TEM) experiments. We suggest here that the low IB thermal conductivity is due to the presence of previously unknown defects in this type of diamond. It is also suggested that these defects are related to the more common 'platelets' or planar faults which are found in type IA diamond.

The thermal conductivity of IB diamond (specimens C8 and C9 in Fig. 1) was first measured in 1972¹ and subsequent experiments^{2,3} have produced similar results. It was found that the strength of the phonon scattering mechanisms which significantly reduced the IB conductivity as compared to purer IIA diamond, could not be explained solely by the presence of paramagnetic singly substitutional nitrogen atoms^{1,4}. This has now been confirmed to be the case for six IB specimens. A satisfactory theoretical explanation of the original measurements on the basis of Rayleigh-type phonon scattering, could not be found⁴ although the most recent results can be explained if the non-paramagnetic nitrogen (which in this case was more highly concentrated than is usual for type IB) was assumed to be in clusters of several atoms⁵.

We chose for our investigations a transparent amber coloured African diamond (no. 2) from a batch provided by the Diamond Research Laboratory on the basis of extremely low birefringence. Electron spin resonance experiments were performed on this specimen using a Varian E-line spectrometer and a characteristic IB response was observed. Singly substitutional nitrogen atoms are responsible for this behaviour⁵. No attempt was made to calculate the spin density rigorously but it was estimated to be $\sim 10^{23} \text{ m}^{-3}$. Infrared and ultraviolet absorption spectroscopy confirmed that the specimen was a typical IB

Fig. 1 The thermal conductivity of two type IB diamonds, ■, C8 and ●, C9. It is impossible to compute the experimental IB thermal conductivity solely by the addition of a phonon point defect scattering term to that used to compute IIA conductivities.



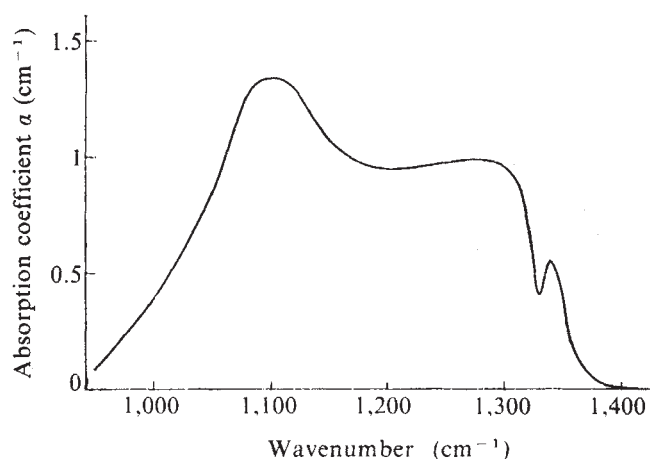


Fig. 2 The infrared absorption spectrum of diamond no 2.

diamond⁶ with an average nitrogen concentration of $\approx 6 \times 10^{24} \text{ m}^{-3}$ depending on what fraction of the $1,280 \text{ cm}^{-1}$ absorption was due to A and B centres (Fig. 2). A thin (100) slice was cut and ground from this diamond and studied further by infrared spectroscopy. It possessed a similar concentration of nitrogen to that of the original cube. A selected area of very low birefringence was thinned by ion beam bombardment⁷ to produce a minute perforation, the edges of which were investigated by TEM. This particular diamond thinned perfectly and particularly quickly ($\sim 3 \mu\text{m h}^{-1}$) thereby creating a larger thickness gradient than usual at the edges of the perforation. The large thickness gradient and perfect surface were responsible for the observation of strong 'thickness' fringes by TEM. Small defects showing as black and white dots were seen near the edges of the dark thickness fringes in a bright-field micrograph (Fig. 3). The white dots were seen on the thin side of the dark fringes, the dark dots were seen on the thin side of the light fringes and no dots were seen near the centre of the fringes. In thicker parts of the foil the effective contrast of the dots was reduced because of increased absorption in the crystal. These observations fit the description of the common type of structure factor contrast usually observed when a small inclusion has a lower structure factor than the matrix⁸. It is debatable whether the defects would be detected in the absence of strong thickness fringes. The strain field of the defect seemed spherical although ill-defined under high resolution (0.2 nm point to point) TEM and typically $\sim 5 \text{ nm}$ in size. They are presumably quite homogeneously distributed in the areas which have been observed by TEM. On this assumption, their concentration was $\sim 2 \times 10^{21} \text{ m}^{-3}$ which is a typical 'platelet' concentration in normal IA material. To ensure that the defects were not artefacts the specimen was re-thinned weakly to remove $\sim 10 \text{ nm}$ of the sample surface and further TEM observations were made. This did not alter the appearance of the defects and we feel, therefore, in the light of previous experience of thinning diamond by ion bombardment, that the defects are real. To ensure that the small diamond slice was IB material a further ESR experiment was performed on the slice alone. A characteristic spectrum was observed which indicated that the slice possessed a similar spin concentration to the original cube.

Further detailed investigation by TEM showed that a small number of these minute defects possessed an appearance characteristic of platelets in IA diamond (Fig. 4). The defects were too small and ill-defined for a definite plane to be assigned to them but $\{100\}$ is consistent with the data. It is suggested that these are minute platelets and that the common defect shown in Fig. 3 is the genesis of such platelets. It is unlikely that the minute platelets are

the genesis of these 'bubbles'. If platelets are subjected to high temperatures and pressures, they form larger structures which have some similarities to giant platelets^{7,9}. Other defects seemed to be at an intermediate stage of development.

It is not possible to determine the exact nature of these defects but voids, bubbles of gas or liquid, amorphous material or crystalline material of a low structure factor are all consistent with the results. It is also possible that the defect could be considerably smaller than 5 nm if a strong strain field is present. Some evidence suggests that hydrogen might be present in the bubbles for, of all the diamonds investigated by ion beam spectrochemical analysis, this IB specimen gave the highest hydrogen content¹⁰.

Presumably if the specimen were subject to an appropriate high temperature and pressure environment the bubbles would transform into minute platelets which could then grow up to $\sim 80 \mu\text{m}$ in length^{7,9,11,12}. It should be noted that the common platelets also have a lower effective scattering factor than the diamond matrix¹³.

These defects or bubbles will act as phonon scattering centres and in an interesting fashion. The dominant phonon wavelength in diamond is equal to the bubble size at a temperature $\sim 100 \text{ K}$. Thus for $T \ll 100 \text{ K}$ one would expect a phonon ω^4 -scattering dependence (Rayleigh scattering) to be most important (where ω is the phonon angular frequency) while for $T \gg 100 \text{ K}$ frequency independent scattering would be prevalent. In the intermediate range ($T \sim 100 \text{ K}$) a complex oscillatory scattering mechanism will be most relevant^{14,15}. Therefore, one would not expect the thermal conductivity of specimens C8 and C9 (Fig. 1) to be explained solely by the addition of ω^4 -Rayleigh scattering if they also contained similar defects to those described here. The magnitude of the phonon scattering associated with these particular defects would be sufficiently high to explain the data on C8 and C9. Generally, if the 'bubble' defect size in IB diamond were smaller ($\lesssim 2 \text{ nm}$) such that Rayleigh-type scattering could adequately describe the phonon defect scattering at all temperatures for which data were available (that is, $T < 300 \text{ K}$), this contribution would be confused with the

Fig. 3 Small defects in a IB diamond showing structure factor contrast. The edge of the specimen is labelled E. Bright-field micrograph. Scale bar $0.1 \mu\text{m}$.



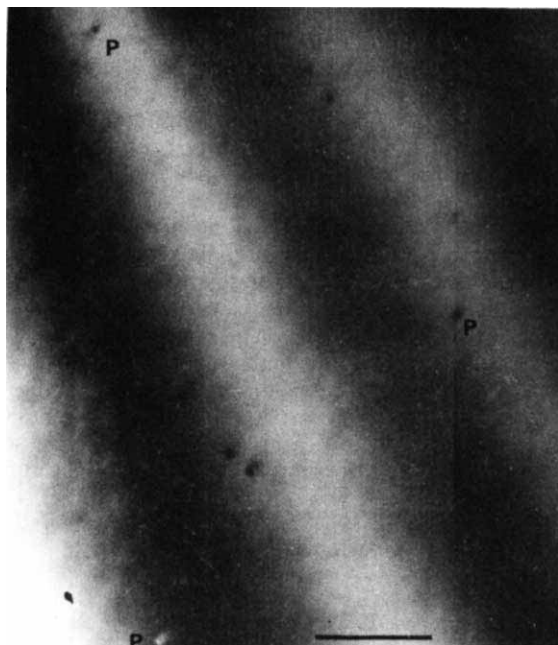


Fig. 4 Small defects in a IB diamond (as used in Fig. 3). Some of these defects appear as minute platelets (P) surrounded by characteristic strain fields. Larger platelets are common in type IA diamond. Scale bar 0.1 μm .

nitrogen cluster scattering mechanism which is identical in its phonon scattering frequency dependence.

We conclude that the thermal conductivity of our sample would be similar to the conductivity of diamonds C8 and C9, and suggest that smaller defects of the same nature might have existed in the other four type IB diamonds for which the thermal conductivity has been measured^{2,3}. It could be that such defects are a general feature of IB diamonds, in which case it would seem that IB diamond is a preliminary state of IA diamond. Synthetic diamond must be the most preliminary state, and a IB diamond will be fabricated if nitrogen is introduced, whereas it has not been possible to synthesise IA diamond¹⁶. If the bubbles contained nitrogen, this would account for the generally higher nitrogen concentration (as detected by infrared) found in IA samples.

P. R. W. HUDSON

P. P. PHAKEY

Physics Department,
Monash University,
Clayton, Victoria 3168, Australia

Received 21 December, 1976; accepted 28 July, 1977.

- ¹ Hudson, P. R. W. *The thermal conductivity of various types of diamond paper presented at Diamond Conf., Bristol* (1972).
- ² Berman, R., Hudson, P. R. W. & Martinez, M. J. *Phys. C: Solid St. Phys.* **8**, L430 (1975).
- ³ Berman, R. & Martinez, M. *Diam. Res.* **7**–13 (1976).
- ⁴ Hudson, P. R. W. thesis, Univ. College, Oxford (1972).
- ⁵ Smith, W. V., Sorokin, P. P., Gelles, I. L. & Lasher, G. J. *Phys. Rev.* **115**, 1546 (1959).
- ⁶ Dyer, H. B., Raal, F. H., Du Preez, L. & Loubser, J. H. N. *Phil. Mag.* **11**, 763 (1965).
- ⁷ Hudson, P. R. W. & Phakey, P. P. *Phys. stat. solid* **a36**, 627 (1976).
- ⁸ Ashby, M. F. & Brown, L. M. *Phil. Mag.* **8**, 1649 (1963).
- ⁹ Evans, T. & Rainey, P. *Proc. R. Soc. A344*, 111 (1975).
- ¹⁰ Hudson, P. R. W. & Tsong, I. S. T. *J. mater. Sci.* (in the press).
- ¹¹ Woods, G. S. *Phil. Mag.* **34**, 993–1012 (1976).
- ¹² Mendelsohn, M. J. thesis, Univ. London (1971).
- ¹³ Bursill, L. A., Barry, J. C., Hudson, P. R. W. (to be published).
- ¹⁴ Schwartz, J. W. & Walker, C. T. *Phys. Rev.* **155**, 969 (1967).
- ¹⁵ Hudson, P. R. W. *Phys. stat. Solid* **a37**, 645 (1976).
- ¹⁶ Woods, G. S. *Diam. Res.* **25**–27 (1973).

Correlation between dielectric constant and defect structure of non-stoichiometric solids

To understand the properties and behaviour of inorganic non-stoichiometric compounds it is vital to know how the stoichiometric imbalance in the material is accommodated structurally. It has been usual to assume that point defect populations have been solely responsible for such departures from the normal stoichiometric formula. But, recent structural investigations have shown that for a growing number of materials this does not seem to be true and instead changes in the anion-cation ratio are accommodated by planar faults. This report points out that an empirical correlation seems to exist between the magnitude of the low frequency dielectric constant of a compound and the structural mode by which it accommodates its non-stoichiometry. Those materials with a low dielectric constant are found to prefer point defect populations, while those with a high dielectric constant prefer planar faults. Some reasons why this correlation should hold are suggested.

Recent studies have mainly led to a recognition of two broad groupings of inorganic non-stoichiometric compounds. First, there are those materials which accommodate stoichiometric changes by way of point defects, either in the classical sense or, in materials with relatively large departures from stoichiometry, by point defect clusters or by ordered arrays of point defects. Examples of this are: Fe_{1-x}O (wüstite), many compounds with the fluorite or fluorite-related structures and the metallic oxides $\text{TiO}_{1\pm x}$ and $\text{VO}_{1\pm x}$ (ref. 1).

The second grouping consists of materials in which any stoichiometric variation seems to be taken up by planar faults or intergrowths. In these materials the point defect contribution to the stoichiometric imbalance seems minimal. Instead the structure of the non-stoichiometric crystal can be regarded geometrically as an intergrowth between two phases which have compatible structures, or else as a parent structure which has collapsed along a particular crystallographic plane so as to eliminate one atom type rather than another from the parent matrix. The intergrowth or fault usually takes the form of a lamella only one or two cation-anion polyhedra thick and hence is related to, but differs in scale, from syntaxy.

As these materials are less well known, some examples will illustrate their behaviour and the range of compounds covered. Perhaps the best documented are the crystallographic shear (CS) phases. These are formed when WO_3 , Nb_2O_5 , TiO_2 and other materials are reduced or doped with (generally) lower valance cations. Characteristically they are slabs of the parent higher oxide containing fault planes, the CS planes, which geometrically are planes along which the structure has collapsed to eliminate oxygen. If these faults are ordered, a homologous series of oxides is formed; typical examples being $\text{W}_n\text{O}_{3n-2}$, $\text{Ti}_n\text{O}_{2n-1}$. More examples of CS phases are given in several reviews (refs 1–3 and refs therein.) A second group consists of oxides often described as intergrowths. In these phases, stoichiometric variation is achieved by the ordered or disordered intergrowth of phases with different compositions. Examples are the perovskite-related oxides formed by intergrowth between NaNbO_3 and complex silicate-related phases $\text{Ba}_3\text{Nb}_2\text{Si}_2\text{O}_{26}$ and $\text{Ba}_3\text{Nb}_2\text{Ti}_2\text{O}_{22}$ (ref. 5). Ordered intergrowths can again be expressed in terms of homologous series formulae. Finally, some of the materials described as polytypes can also be placed into this category. Of particular note are the hexagonal ferrites⁶ where structural complexity is associated with intergrowth between units of the spinel structure. The titanium sulphides with compositions lying near to Ti_2S_3

Table 1 Dielectric constant and defect structure type of some non-stoichiometric compounds

Material	Dielectric constant*	Frequency (Hz)	Non-stoichiometry†
FeO	14.2	2×10^6	P.D.
NiO	11.9	10^6	P.D.
CaO.ZrO ₂	~12.0	2×10^6	P.D.
CaF ₂	6.8	10^3	P.D.
UO ₂	24	3×10^5	P.D.
ThO ₂	18.9	3×10^5	P.D.
CeO ₂	7.0	2×10^6	P.D.
V ₂ O ₅	~15 (11)	~ 10^6	P.D. (15)
WO ₃	~ 10^3 (12)	~ 10^6	CS
TiO ₂	$\epsilon_c = 167$ (13)	~ 10^6	CS
H-Nb ₂ O ₅	~200 (14)	~ 10^6	CS
SrTiO ₃	~350	10^3	I (16)
PbS	~200	i.r.	I‡
Bi ₃ Ti ₃ O ₁₂	~200	10^3	I
NaNbO ₃	$\epsilon_{33} \sim 670$	—	{ I (4)
Ca ₂ Nb ₂ O ₇	~45	5×10^7	

*Values are room temperature values, taken from Young and Frederikse (ref. 10), or the references in parentheses.

†P.D., ordered, clustered or random point defects; CS, crystallographic shear; I, intergrowth. Fuller descriptions of defect type will be found in refs 1–3 or the references in parentheses.

‡A. N. Bagshaw, personal communication.

also fall into this group. Stoichiometric variation here can be described as being due to intergrowth between the TiS phase and the TiS₂ phase⁷.

For evaluating materials growth methods, or predicting the chemical or physical behaviour of materials it is valuable to have a reliable guide as to which of these modes of stoichiometric behaviour is likely to predominate in any particular system but at present no such guide has been found.

Calculations of the electrostatic interaction between CS planes in reduced WO₃ (ref. 8) showed that a high value of the low frequency dielectric constant was of importance in reducing this energy term to a small value. The fact that CS planes only seemed to be found in materials which possessed a high dielectric constant was also pointed out by Bursill *et al.*⁹. Taking these two observations as starting points, I have examined the literature in an attempt to determine whether there is a definite correlation between the occurrence of intergrowth as a means of accommodating non-stoichiometry in a material and low frequency dielectric constant. Although the data on both these topics are scanty a very good correlation is found, provided that those materials in which a high value of the low frequency dielectric constant is associated with hydrogen bonding are excluded, as the crystal-chemical relationship between dielectric constant and structure is rather different in these compounds than in, for example, the perovskite oxides.

Table 1 reports some materials with well known modes of accommodating non-stoichiometry which also have recorded dielectric constant values. Unfortunately data are not available for many interesting compounds, but there are enough to show that systems using point defects have low values of dielectric constant, less than approximately 24, while all the materials with known intergrowth or CS behaviour have values >100.

In order to apply this empirical correlation usefully it is important to remember that most experiments involving non-stoichiometric behaviour are carried out at moderate to high temperatures. The room temperature dielectric constant which is the value most usually reported and which

is given in Table 1 may well be considerably lower than that at high temperatures, and temperature variation can be large, as in the case of WO₃ (ref. 12). Thus for some materials, a change in behaviour with temperature may be expected, MoO₃ may be such an example¹⁷.

In the non-metallic materials we are considering, the low frequency dielectric constant is composed of two components, the electronic and ionic polarisabilities of the atoms in the structure. A high value of the static dielectric constant is often attributable to a high ionic polarisability term, which is largely due to the presence of small off-centre cations in metal-oxygen coordination octahedra which form the building units of many oxides. The ferroelectric perovskites fall into this class. In other materials, such as PbS or SbSI the presence of large easily polarisable anions and lone pairs of electrons on the cations produces large electronic polarisation. Neither of these features in themselves, however, seem to explain why a point defect population should be resisted in these materials, although recent lattice energy calculations for materials containing point defects have shown that polarisation terms have a considerable effect on defect formation energies.

A possible reason for the rejection of high point defect populations may be found by considering the lattice dynamics of crystals. The low frequency dielectric constant of a crystal and hence the ionic and electronic polarisabilities of the component atoms are closely connected with the allowed lattice vibrations of the matrix. The simplest form this relationship takes is expressed by the Lyddane-Sachs-Teller relationship which shows that a high static dielectric constant is associated with a transverse optical phonon, ω_T , of very low frequency in the lattice¹⁸. These soft transverse modes are temperature dependent and tend to exhibit instability as the frequency falls, that is, as the dielectric constant increases. Barker¹⁹ has shown that soft mode instability can be strongly influenced by the local electric field within a dielectric material. The presence of defects in a material, either some form of point defect arrangement or intergrowths or planar faults, will lead to significant changes in the local fields in some regions of the crystal which will have a bearing on the stability of the soft ω_T modes. The interaction will almost certainly be complex and has not been analysed quantitatively, but it seems reasonable to suggest that in materials with a high dielectric constant the existence of potentially unstable soft ω_T modes favours the formation of sheets of defects, intergrowths or crystallographic shear planes, over point defects or point defect clusters.

The lack of a detailed theoretical treatment, together with the paucity of experimental data make these suggestions very speculative. Despite these qualifications the empirical correlation between dielectric constant and defect structure seems to work well and be useful. It provides a guide to the structural behaviour of non-stoichiometric solids where none has existed before. Theoretical studies are now underway which, it is hoped, will provide a more quantitative understanding of the problem. In particular it is important to determine the extent to which there may be a causal relationship between low frequency dielectric constant and the mode of accommodation of non-stoichiometry which, at present, is by no means certain.

R. J. D. TILLEY

School of Materials Science,
University of Bradford,
Bradford,
W. Yorkshire, UK

Received 23 June; accepted 25 July 1977.

- ¹ Anderson, J. S. in *Defects and Transport in Oxides* (eds Seltzer, M. S. & Jaffee, R. I.) 24–54 (Plenum, New York, 1974).
- ² Anderson, J. S. & Tilley, R. J. D. in *Surface and Defect Properties of Solids 3*, (eds Roberts, M. W. & Thomas, J. M.) 1–56 (Chemical Society, London, 1974).
- ³ Hyde, B. G., Bagshaw, A. N., Anderson, S. & O'Keeffe, M. in *A. Rev. mater. Sci.* 4, 43–92 (1974).
- ⁴ Nanot, M., Queyreux, F., Gilles, J. C., Portier, R. & Fayard, M. *Mater. Res. Bull.* 10, 313–316 (1975).
- ⁵ Nguyen, N., Studer, F., Groult, D., Choynet, J. & Raveau, B. *J. Solid St. Chem.* 19, 369–382 (1976).
- ⁶ McConnell, J. D. M., Hutchison, J. L. & Anderson, J. S. *Proc. R. Soc. A* 339, 1–12 (1974).
- ⁷ Tilley, R. J. D. *J. Solid St. Chem.* 7, 213–221 (1973).
- ⁸ Iguchi, E. & Tilley, R. J. D. *J. Solid St. Chem.* (in the press).
- ⁹ Bursill, L. A., Hyde, B. G. & O'Keeffe, M. in *Solid State Chemistry*, N. B. S. spec. Pub. 364, (eds Roth, R. S. & Schneider, S. J.) 197–204 (National Bureau Standards, Washington, 1972).
- ¹⁰ Young, K. F. & Fredericks, H. P. R. *J. phys. Chem. Ref. Data* 2, 313–409 (1973).
- ¹¹ Chernko, I. M. & Ivon, A. I. *Fiz. Tverd. Tela* 16, 2130–2133 (1974); English translation, *Sov. Phys. Solid St.* 16, 1391 (1975).
- ¹² Leikowitz, I., Dowell, M. B. & Shields, M. A. *J. Solid St. Chem.* 15, 24–39 (1975).
- ¹³ Samara, G. A. & Percy, P. S. *Phys. Rev. B* 7, 1131–1148 (1973).
- ¹⁴ Emmenegger, F. P. & Robinson, M. L. A. *J. phys. Chem. Solids* 29, 1673–1681 (1968).
- ¹⁵ Hyde, B. G. & Tilley, R. J. D. *J. phys. Chem. Solids* 31, 1613–1619 (1970).
- ¹⁶ Tilley, R. J. D. *J. Solid St. Chem.* (in the press).
- ¹⁷ Bursill, L. A. *Proc. R. Soc. A* 311, 267–290 (1969).
- ¹⁸ Peterson, G. E. in *Treatise on Solid State Chemistry* 2 (ed. Hannay, N. B.) 183–236 (Plenum, New York, London, 1975).
- ¹⁹ Barker, A. S. in *Ferroelectrics* (ed. Weller, E. F.) 213–250 (Elsevier, Amsterdam, 1967).

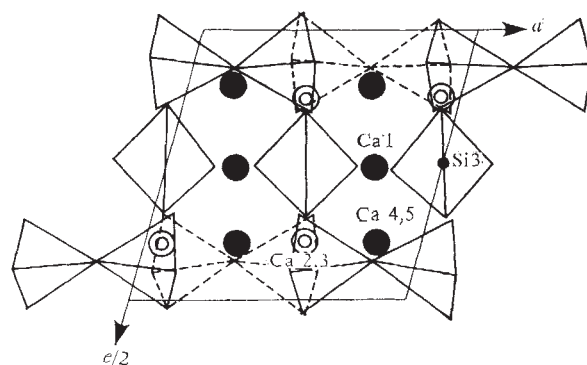


Fig. 1 Rustumite—(O10) projection of one half of the unit-cell. The Si_2O_7 and SiO_4 groups are indicated as polyhedra. The Ca atoms overlap in pairs: ●, (Ca1, Ca1'), (Ca4, Ca5); ○, (Ca2, Ca3).

Crystal structure of rustumite

RUSTUMITE was first described by Agrell¹ as $\text{Ca}_4\text{Si}_2\text{O}_7(\text{OH})_2$, space group Cc or C2/c, $a = 7.62(5)$, $b = 18.55(5)$, $c = 15.51(5)$ Å, $\beta = 104^\circ 20'(10')$, with $Z = 10$. The crystal structure we describe here, in space group C2/c ($R = 11.3\%$ for all data) gives, instead, an ideal formula $\text{Ca}_{10}(\text{Si}_2\text{O}_7)_2\text{SiO}_4\text{Cl}_2(\text{OH})_2$, with $Z = 4$. The important features of the completed structure are the presence of chlorine and of orthosilicate groups in addition to Si_2O_7 groups.

The experimental intensity data, from a naturally occurring single crystal, comprised 2,100 independent reflections for layers 0–9, k, l ($\sin^2\theta < 0.2$) obtained with a Hilger and Watts Y-190 linear diffractometer and Mo- $K\alpha$ radiation, using the cell dimensions given by Agrell¹. Of this data, 282 reflections were classed as 'unobserved', and were excluded from all further calculations.

Solution of the Patterson map was hindered by the large number of atoms in the unit cell (~ 150), permitting the detection of only four (or more)-multiple peaks, and the presence of subtranslations of $\sim a/4$, $\sim b/6$ and $\sim c/6$ among the Patterson

peaks. Nevertheless, an initial structure was established in the space group C1 by double superposition using two multiple peaks as shift vectors² and consideration of the vector sub-system³.

In the course of refinement by the usual Fourier methods, as the remaining atoms of the structure appeared, the presence of first the twofold axis and finally the c -glide became obvious. The final solution in the space group C2/c, refined by block diagonal least squares using all of the observed reflections except 171 which showed especially poor agreement between F_o and F_c , gave the parameters of Table 1. At this stage R was 7.4% for the data included in least squares (11.3% over all observed data).

In the asymmetric unit it is easy to pick out, apart from the five Ca atoms, an $(\text{Si}_2\text{O}_7)^{6-}$ group (involving Si1 and Si2) and an $(\text{SiO}_4)^{4-}$ group involving Si3 on a twofold axis (Fig. 1), leaving only O10 and O11. The atom O10 yields a peak in the electron density maps comparable in size with Si3, gives an occupancy factor (refined as oxygen) of ~ 2.0 in least squares refinement and has no nearest-neighbour Ca atom closer than 2.75 Å, all consistent with this atom being Cl. Bogomolov and Organova (personal communication) have given analytical data for chlorine in rustumite.

Whereas all other O atoms, including O10, are four-coordinate, O11 has only three Ca nearest neighbours (Ca—O, 2.33–2.37 Å) all to one side of it, clearly allowing completion of its tetrahedral coordination by a hydrogen atom. Therefore, although there is no clear evidence for the H atom in the electron density or difference maps, O11 can reasonably be regarded as a hydroxyl group to give the formula $\text{Ca}_5(\text{Si}_2\text{O}_7)(\text{SiO}_4)_2\text{Cl}(\text{OH})$ for the formula of the asymmetric unit (eight per cell) of rustumite. The cell contents are then $\text{Ca}_{40}\text{Si}_{20}\text{O}_{72}\text{Cl}_8(\text{OH})_8$ compared with $\text{Ca}_{40}\text{Si}_{20}\text{O}_{70}(\text{OH})_{20}$ from Agrell's formula.

We thank Professor H. F. W. Taylor for bringing this problem to our attention and the SRC for a grant to V.V.I.

R. A. HOWIE

V. V. ILYUKHIN*

Department of Chemistry,
University of Aberdeen,
Meston Walk,
Aberdeen, UK

Received 1 July; accepted 28 July 1977.

*Permanent address: Institute of Crystallography, Academy of Science, Moscow, USSR.

¹ Agrell, S. O. *Min Mag.* 34, 1–15 (1965).

² Ilyukhin, V. V. *Direct and Patterson Methods for Solving Crystal Structures* (in Russian, Institute of Applied Physics, Academy of Science (Moldavia), U. S. S. R., Kishinev, 1972).

³ Kuz'min, E. A., Ilyukhin, V. V. & Petrunina, A. A. *The Method of Vector Sub-Systems* (in Russian, Institute of Geochemistry and Mineral Physics, Academy of Science (Ukraine), U. S. S. R., Kiev, 1976).

Atom	Fractional coordinates			B_{iso} Å ² (10 ²)	Occupancy (10 ²)
	x/a	y/b	z/c		
Ca(1)	2,582(4)	3,520(1)	2,498(2)	95(4)	98(1)
Ca(2)	4,399(4)	3,024(1)	6,192(2)	96(4)	97(1)
Ca(3)	5,760(4)	2,068(1)	4,039(2)	95(4)	96(1)
Ca(4)	3,298(4)	232(2)	4,017(2)	102(4)	98(1)
Ca(5)	3,106(4)	5,148(1)	3,964(2)	93(4)	97(1)
Si(1)	4,420(5)	6,319(2)	5,614(2)	76(5)	98(1)
Si(2)	3,692(5)	1,340(2)	5,643(2)	83(5)	99(1)
O(1)	3,349(12)	3,786(5)	4,161(6)	99(16)	93(2)
O(2)	4,782(12)	1,759(5)	6,518(6)	96(15)	98(2)
O(3)	4,318(12)	523(5)	5,559(6)	118(15)	98(2)
O(4)	4,629(12)	5,760(5)	1,594(6)	95(15)	97(2)
O(5)	3,503(13)	1,770(5)	4,725(6)	110(17)	89(2)
O(6)	3,767(13)	6,870(5)	4,777(6)	105(16)	93(2)
O(7)	4,236(13)	3,277(5)	1,516(6)	136(16)	98(2)
O(8)	3,168(13)	4,778(5)	2,481(6)	107(15)	97(2)
O(9)	3,695(13)	4,476(5)	429(6)	119(16)	94(2)
O(10)	2,384(6)	2,961(2)	7,458(3)	54(6)	204(2)
O(11)	560(14)	5,881(5)	3,535(6)	123(17)	91(2)
Si(3)*	1/2	5,276(3)	1/4	82(8)	97(2)

Estimated standard deviations applicable to the least significant digits are given in parentheses.

*Si(3) is in the special position 4e of C2/c, while all other atoms occupy general (8f) positions.

Imaging practical surfaces in a field ion microscope

APPLICATIONS of the field ion microscope (FIM), have been restricted probably because of the need for specially prepared specimens. We present here, for the first time, experimental data on imaging practical surfaces with the FIM. This should provide more applications for this instrument especially in studying multi-protrusions or whiskers.

The techniques used in the FIM including the vacuum system are conventional. In the first stage of the experiments, we tried imaging multi-tips from a single tip holder. Surprisingly, we could identify the individual images of the single tips, and these were geometrically superposed. The images of twin tips of tungsten are shown in Fig. 1a. Figure 1b shows a simulated pattern obtained by superposing two negatives of a single tip image of tungsten. The close correspondence between the two patterns is striking. This imaging was repeated for three and so on up to seven tips. In each case it was possible to get the resolved images although their stability was poor because of the unavoidable differences in specimen tip size, shape and relative displacements, all of which led to a vast difference of field concentrations about each tip.

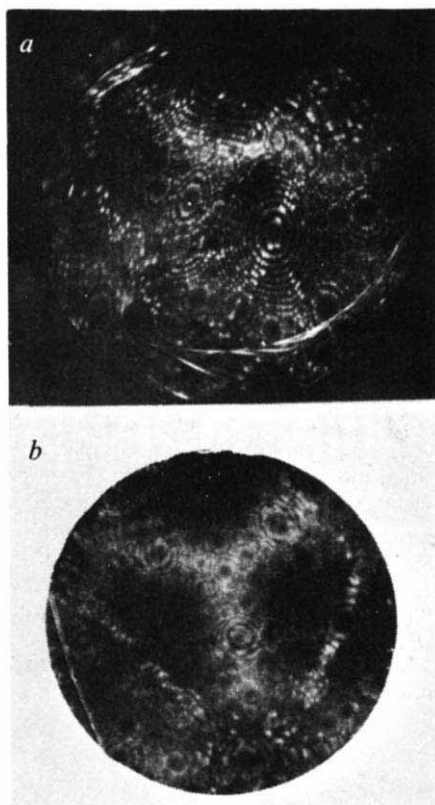


Fig. 1 a, Twin tips of tungsten imaged. b, Superposed images of two tungsten tips. Voltage, 17 kV.

In each case, another striking observation was made after the tips were blunted by rapid field-evaporation. Even at the highest voltage of 28 kV (limited by our high voltage unit), no resolved image could be seen. In all these cases, however, the image pattern exposed bright circles—usually rings with haloes around them—the number corresponding to the number of multi-tips mounted for the projection (Figs 2, 3). Using scanning electron microscope (SEM) patterns for these tips, and assuming an approximate field-factor, the magnification for these tips was estimated and a good experimental fit was observed. But the reason for

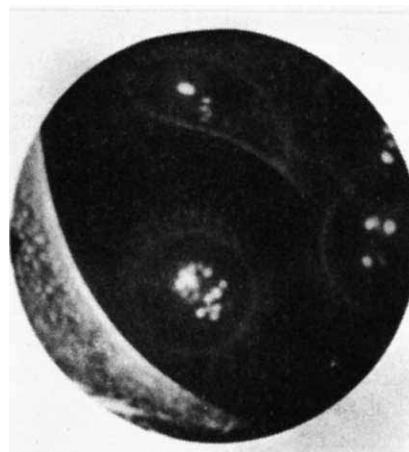


Fig. 2 Image of four tips depicting four rings. Voltage, 25 kV.

these bright ring images is not clear. As tips were blunt, the available voltage was probably insufficient to obtain best imaging voltage (BIV) with helium gas. All imaging was done at background pressure of 10^{-6} torr or less. At the liquid nitrogen temperatures at which our voltages were applied, nitrogen and other residual gases probably reached the tip by surface migration along the shank¹, and then field-

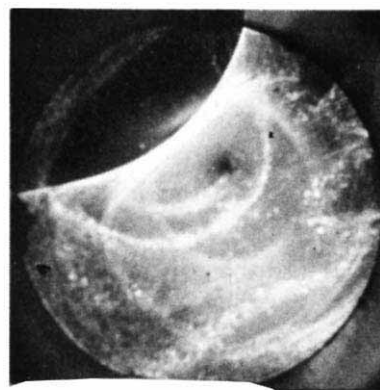


Fig. 3 Image of seven tips depicting seven rings. Voltage, 42 kV.

evaporated. Did the dimension of the ring, then, represent the tip diameter? If the relative displacements of these tips could be assumed, then these images would help trace the electric field contours and field-factor determination for these complex conditions. At times, one could even observe very strange cross lines (Fig. 4) which can only be understood as representing the arc of field distribution which

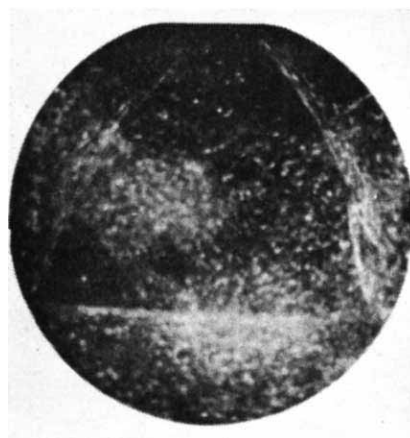


Fig. 4 Cross lines depicting potential profile. Voltage, 45 kV.

would be too complicated to map for the multiple tips case where equipotential overlappings are expected.

In a later experiment carried out on three tip specimens, an interesting feature was observed. After the first flashings and bluntings of the tips, one microprotrusion, probably belonging to one of the blunt tips became very well resolved at a voltage of 32 kV. When the imaging gas helium was cut out, the resolved tip image disappeared completely leaving the surrounding random adsorption pattern unchanged (Fig. 5*a, b*). This resolved image could also be field-evaporated later atom by atom in a controlled fashion until the image completely vanished. The protrusion size calculated from the evaporated rings was 21 Å.

Our most interesting results were obtained when a good low voltage single tip (Fig. 6) slipped and fractured during rapid field-evaporation. On raising the voltage, distinct circles were seen (Fig. 7), representing different microprotrusions created on the main tip by the fracture. One ring was seen as distinctly separated from an overlapping group (estimated to be four) of rings. At a higher voltage,

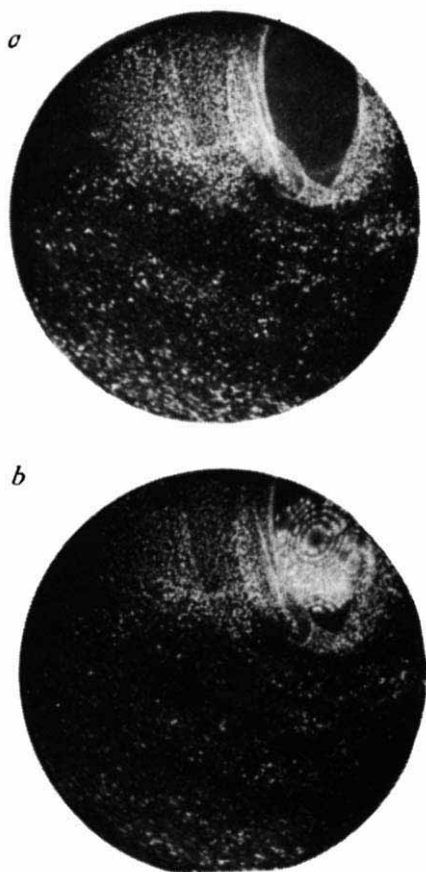


Fig. 5 *a*, Image of deformed tip without imaging gas. *b*, Image of the same tip with helium gas. Voltage, 32 kV.

~41 kV, this group of overlapping rings resolved (Fig. 8) and at least one well defined symmetric tungsten image could be clearly detected. This microprotrusion could be clearly field-evaporated and imaged without any interference. The radius of the original tip was calculated using both Bowkett's² and Müller's³ methods and a value of 220 Å was obtained. By a similar method, the radius of the microprotrusion was calculated to be 35 Å. Using Rose's⁴ formula for magnification enhancement for a conducting protrusion on a field emitter, we calculated the magnification expected for our micro-tip and, finally, its image diameter as observed on the screen. The calculated diameter was about 4 cm and the experimentally observed image was a ring 3.7



Fig. 6 Single tip of tungsten. BIV, 9.8 kV.

cm in diameter. The SEM pattern taken at the end of this experiment did not indicate the presence of a protrusion. This renders our technique more sensitive for the detection of such extremely small protrusions unresolved by other microscopic techniques available.

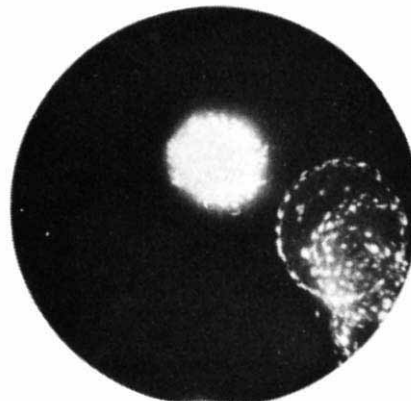


Fig. 7 Tip of tungsten (the same as in Fig. 6) after a flash. Voltage, 18 kV.

To conclude, it is possible to resolve individual microprotrusions of a few tens of ångströms on a practical surface using the field ion microscope.

Further it seems feasible to (1) map equipotentials for complex distribution of conducting protrusions on a surface and calculate field factors which hitherto was not possible;

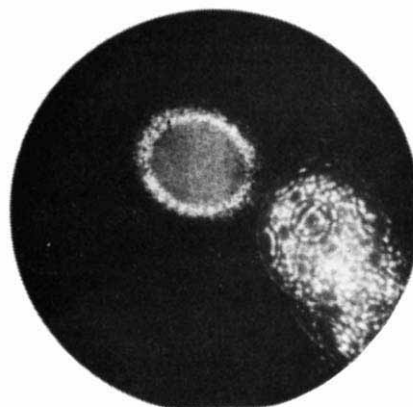


Fig. 8 Resolved microtip. Voltage, 43 kV.

and (2) image simultaneously and without mutual interference, dissimilar elements that can be grown as micro-protrusions or whiskers. This will enable us to understand specific sites on the substrate for nucleation, in relation to crystallographic features and so on. The atom probe⁵ coupled to this mode of operation of FIM will permit us to chemically identify individual atoms from different protrusions.

We are grateful to the late Dr D. Y. Phadke who encouraged this unconventional approach.

P. VIJENDRAN

DEVAKI RAMANATHAN

SUNDER DASS

Technical Physics Division,
Bhabha Atomic Research Centre,
Bombay 400 085, India

Received 10 May; accepted 25 July 1977.

- ¹ Muller, E. W. *Field Emission Studies of Metal Surfaces* 47 (Tech. Rep. AF 49 (638)—504 Solid State Division Air Force Office of Scientific Research, Washington, 1961).
- ² Bowkett, K. M. & Smith, D. A. *Field Ion Microscope* 37–39 (North Holland, Amsterdam, 1970).
- ³ Muller, E. W. & Tsong, T. T. *Field Ion Microscopy—Principles and Applications* 187–189 (Elsevier, New York, 1969).
- ⁴ Rose, D. J. *J. appl. Phys.* 27, 215 (1956).
- ⁵ Muller, E. W. *Lab. Pract.* 22, 408–415 (1973).

Archaeological evidence for Subrecent seismic activity along the Dead Sea–Jordan Rift

EXAMINATION of archaeological sites and records in the Levant reveals features of destruction attributable to ancient earthquakes, which may help in verification of old earthquake catalogues and in assessment of seismic hazards.

Regional seismicity in Israel is characterised by sporadic shocks of up to $M=7$, separated by long quiescent periods. The shocks, believed to originate mainly along the Dead Sea–Jordan Rift, where microtremors occur also during the quiescent periods, are documented by instrumental and historical data^{1–7}. Historical earthquake evidence, however, is based on biblical, ecclesiastical and historical chronicles, which are tainted by superstition, exaggeration and misinterpretation of natural phenomena. Consequently, the estimates of incidence, intensity and distribution of individual shocks presented by different cataloguers differ considerably^{1–6,8,9}.

To obtain material evidence¹⁰, we have examined the record of archaeological excavations in Israel, searching for reports of catastrophic damage at explored sites. Summaries of archaeological findings^{11,12} and responses to questionnaires circulated among Israeli archaeologists revealed numerous cases in which destruction and desertion were attributed to major earthquakes (Table 1). Field examination of the reputed features of seismic damage (for example, joints, cracks, faults and breakage; tilted and distorted walls, buildings and aqueducts; oriented collapse and subsidence; and parallel alignment of fallen columns and masonry) showed them to be less unequivocal than generally assumed by archaeologists, because very similar effects may be caused by poor construction or unfavourable geotechnical conditions, unrelated to any seismic events. At the same time, it seems that these 'earthquake indicators' were used by archaeologists fairly consistently, and that the assignment of such features of architectural damage to seismic shocks at

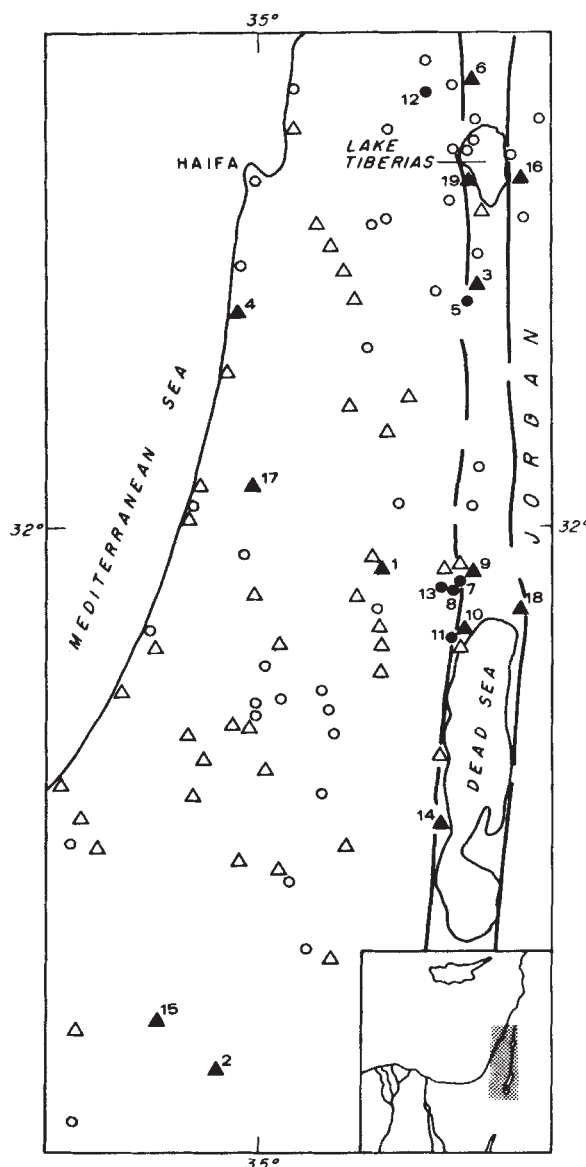


Fig. 1 Distribution of suspected pre-Crusader archaeoseismic damage in Israel. ○, Sites with one or two exposed buildings. △, Sites with numerous exposed buildings and structures. ●, ▲, Sites with reported archaeoseismic damage. Numbers refer to locations presented in Table 1. Heavy lines: major Rift faults.

any individual site, though probably prejudiced by interpretation of historical records, was unbiased by previous geological or geotechnical considerations.

Of all the excavated pre-1000 AD (pre-Crusader) sites, which are fairly evenly distributed over northern and central Israel, although sparser in the more arid south, we plotted in Fig. 1 only those where excavations uncovered building elements of extent and quality sufficiently high for features of architectural damage not to pass unnoticed. On the same map we marked all the sites with supposed seismic damage. The shock-affected sites differ in lithological and topographical setting, and most of them are located along the Dead Sea–Jordan Rift and its margins. This concentration (about 75%) is greater than that indicated in the historical catalogues (15–30% of references mention the Rift zone). The present distribution of these sites does not show whether they are located along the major Rift faults or along subsidiary crescentic faults and blocks or both. It

Table 1. Reports of ancient seismic damage at archaeological sites in Israel

Location number (Fig. 1)	Site	Evidence	Age of damage	Reference
1	Ai	Widespread destruction, collapse of buildings, breakage, tilting	27th century BC	11, 12
2	Avdat (Eboda)	Damaged city walls and buildings, cracks, subsequent repairs	5–6th century AD	11, 12
3	Beit Shean	Disturbances and collapse in the northern cemetery	?	11, 12
4	Caesarea	Disturbed offshore moles and port installations, tilted walls	2nd century AD	11, 12
5	Ein Hanaziv	Oriented collapse, alignment of fallen masonry	7th century AD	13
6	Hazor	Tilted columns, walls and buildings	8th century BC	11, 12
7	Jericho-Tel el Sultan	Collapse and slides	?	11, 12
8	Jericho-Tel Abu Alaik	Tilted and distorted walls, collapse, subsidence and breakage	1st century BC	14
9	Jericho-Khirbet el Mafjar	Destruction and collapse	8th century AD	11, 12
10	Khirbet Qumran	Displacement and breakage, cracks	1st century BC	11, 12
11	Khirbet Magari	Violent destruction, collapse	7–8th century BC	11, 12
12	Khirbet Shama	Collapse, tilted and distorted (?) walls	4–5th century AD	15
13	Kypros	Collapse, imbricated fallen masonry	?	Mesheh (unpublished)
14	Massada	Disturbed floors	1st century BC	11, 12
15	Shivta	Tilted walls, aligned fallen masonry, cracks, collapse	1st century BC and later shocks	11, 12
16	Susita	Damaged buildings and collapse	5–6th century AD	11, 12
17	Tel Afeq (Antipatris)	Tilted buildings and walls, aligned fallen columns	?	11, 12
18	Tel Afeq (Antipatris)	Tilted and distorted walls, breakage, subsided arches	5th century AD	16
19	Teleilat Ghassul	Tilting, faulting, cracks, subsidence and collapse	40th century BC	17, 18
19	Tiberias	Desertion of the southern city	11th century AD	Foerster (personal communication)

seems to show, however, that the Rift region is seismogenic and that the seismic hazards here are relatively high.

A part of this study was aided by grants to I. K. from the US Academy of Sciences (Day Fund) and the SUNY Faculty Awards Committee. The advice of M. Ben Dov, D. Bahat, M. Kochavi, Y. Mincker, E. Nezer and F. Vitto is gratefully acknowledged.

IAAKOV KARZ

Department of Geology,
SUNY Binghamton,
Binghamton, New York 13901
and Geological Survey of Israel

URI KAFRI

Geological Survey of Israel,
Jerusalem, Israel

ZEEV MESHEL

Institute of Archeology,
Tel Aviv University,
Tel Aviv, Israel

Received 16 May; accepted 5 July 1977.

- Willis, B. *Bull. seism. Soc. Am.* **18**, 73–103 (1928).
- Sieberg, A. *Denkschriften Mediz. Naturwiss. Gesellschaft zu Jena* **18**, 2, 161–273 (1932).
- Shalem, N. *Jerusalem* **2**, 1–3, 22–54 (1949) in Hebrew.
- Amiran, D. *Israel Expl. J.* **1**, 223–246 (1951); 48–65 (1952).
- Shalem, N. *Bull. Res. Coun. Israel* **2**, 2, 1–16 (1952).
- Ben Menahem, A., Nur, A. & Vered, M. *Phys. Earth planet Int.* **12**, 1–50 (1976).
- Wu, F., Karcz, I., Arie, E., Kafri, U. & Peled, U. *Geology* **1**, 159–161 (1973).
- Ambraseys, N. *Nature* **232**, 375–379 (1971).
- Ambraseys, N. in *Historical Geography of the Middle East* (ed. Brice, W.) (Academic, London, 1975).
- Ambraseys, N. *Antiquity* **47**, 229–230 (1973).

¹¹ *Encyclopaedia of Archeological Excavations in Eretz Israel*, Hebrew edn, 2 vols (Massada, Jerusalem, 1970).

¹² *Encyclopaedia of Archeological Excavations in Eretz Israel*, English edn (updated), vols 1 and 2 (from 4) (Massada, Jerusalem, 1975).

¹³ Vitto, F. *Qadmoniot* **8**, 119–123 (1975).

¹⁴ Nezer, E. *Israel Expl. J.* **25**, 89–100 (1975).

¹⁵ Meyers, E. M., Kraebel, A. T. & Strange, J. F. *Israel Expl. J.* **22**, 174–175 (1972).

¹⁶ Kochavi, M. *Israel Expl. J.* **26**, 51–53 (1976).

¹⁷ North, R. *Analecta Biblica* **14**, 1–88 (1960).

¹⁸ Hennessey, I. B. *Levant* **1**, 1–25 (1969).

Bacterial sulphate reduction and calcite precipitation in hypersaline deposition of bituminous shales

BITUMINOUS shales of the Ghareb Formation of Maastrichtian age from Nebi Musa (near Jericho, Jordan Valley) were analysed for their texture, mineralogical and chemical composition. The results from the lowermost bed of the formation are of broad interest, and indicate special diagenetic processes affecting sediments rich in organic material deposited in an anaerobic hypersaline environment. Single crystal calcite replacement of gypsum infilling of foraminifera and a predominance of even over odd carbon numbers among C₂₈–C₃₀ n-alkanes indicate sulphate reducing bacterial activity during deposition of bituminous shales in a hypersaline environment.

The samples taken consist of hard black calcareous shale with up to 15% organic carbon, containing also kaolinite, detrital phosphate and pyrite. Foraminifera constitute a major component—particularly thin walled, small plank-

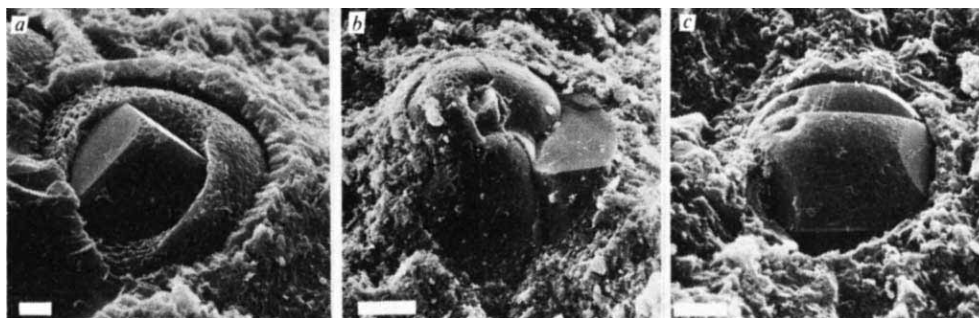


Fig. 1 Scanning electron micrographs of calcite replacement of gypsum infilling of foraminiferal tests. *a*, Chamber filled with gypsum (pitted surface) and a calcite crystal growing in its centre. Scale bar 5 μm . *b*, Chamber partially filled with a euhedral single calcite crystal. Scale bar 5 μm . *c*, Foraminifera completely filled with single calcite crystals. Note the preservation of the original thin walls. Scale bar 50 μm .

tonic ones. The benthonic foraminiferal fauna is composed exclusively of numerous specimens of Buliminidae of one or two species only. Exclusively benthonic Buliminidae assemblages occurring in the phosphates of the underlying Mishash Formation were interpreted as indicating semi-closed, near-shore basins with low surface salinity and oxygen deficiency¹. The addition of abundant planktonic foraminifera to the Buliminidae assemblages in the Ghareb Formation may indicate a change of salinity in the surface waters. The organic matter (mostly kerogen) is dispersed and no structure indicative of its source can be observed even in the scanning electron microscope (SEM).

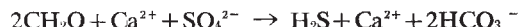
A mineralogical investigation using petrographic microscopy, X-ray diffraction and microprobe analysis revealed that the foraminiferal tests are filled with either of the following minerals: gypsum, single crystals of calcite, and pyrite, which is also dispersed in the matrix. The calcite infilling in the form of a single crystal in a foraminiferal chamber or a whole test was observed optically under the petrographic microscope and morphologically in the SEM. Locally, stages in the replacement of gypsum by single crystal calcite growth can be observed with the SEM (Fig. 1*a*), as well as steps towards complete single crystal calcite infilling of a foraminiferal test (Fig. 1*b*, 1*c*). This infilling cannot be regarded as a late diagenetic sparry calcite mosaic from which it differs in texture. Moreover, sparry calcite development through dissolution and precipitation is not likely to have taken place in the impervious shales.

The samples from the lower Ghareb Formation showing these mineralogical features also exhibit particular organo-geochemical markers, especially with regard to the *n*-alkanes extracted. There is a high concentration of *n*-C₁₉ alkane and a prominent envelope in the C₂₆–C₃₂ carbon number range, in which even carbon number C₂₈ and C₃₀ predominate over their odd carbon number homologues (Fig. 2*b*). This *n*-alkane distribution differs from that of living and fossil higher plants, which are characterised by a prominent envelope in the C₂₃–C₃₃ range, with a marked odd over even preference^{2,3}, as well as from that of algae and photosynthetic bacteria marked by a higher concentration around *n*-C₁₇ (refs 4–6), such as is found in higher strata of this formation (Fig. 2*a*).

Even over odd preference in the C₂₆–C₃₂ range in rock extracted *n*-alkanes was attributed to highly saline environments mainly on the basis of local geological and sedimentological considerations^{7,8}. Studies of the *n*-alkane content of algae and bacteria grown in the laboratory showed that non-photosynthetic anaerobic bacteria have a high concentration of *n*-C₁₉ and longer chain alkanes^{4,5}. An even over odd preference in the C₂₆–C₃₂ range of *n*-alkanes was reported from cultures of *Desulfovibrio hildenboroughi*⁴, a common sulphate reducing bacterial species. It seems, therefore, that the *n*-alkane distribution of the samples investigated is indicative of contributions from both anaerobic, non-photosynthetic, as well as sulphate reducing bacteria.

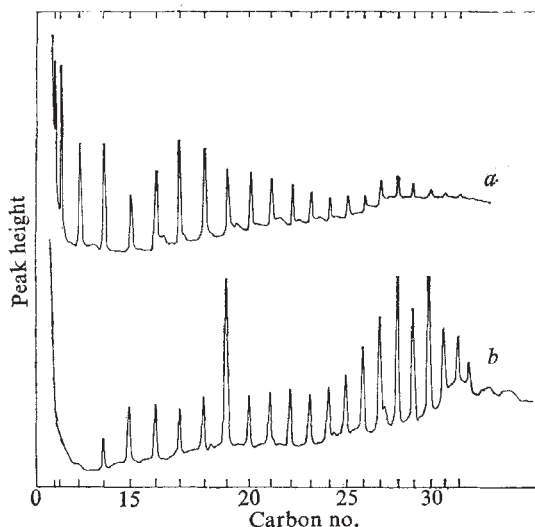
The palaeontological and sedimentological record of the lowermost beds of the Ghareb Formation, the occurrence of gypsum infillings of foraminiferal tests, the single crystal calcite replacement of gypsum, as well as the peculiar *n*-alkane distribution which resembles that of anaerobic and sulphate reducing bacteria, can all be interpreted in terms of environment of deposition and early diagenetic processes as follows.

(1) The bituminous shales were deposited on the bottom of a hypersaline, stratified lagoon, the upper oxygenated, highly productive layer of which was rich in planktonic foraminifera, mainly in the outer part of the lagoon. Organic matter accumulated on the anoxic bottom. (2) Empty foraminiferal tests transported to the inner margin of the lagoon were filled with gypsum either in the upper layer or on the bottom immediately after settling. (3) Anaerobic non-photosynthetic and sulphate reducing bacterial activity resulted in dissolution of gypsum according to the generalised formula⁹



thus increasing the bicarbonate and calcium ion concentration. (4) The waters of the lagoon were already oversaturated with regard to calcium carbonate, therefore, calcite precipitation took place concomitantly where gypsum was dissolved. (5) The presence of pyrite is at least partly due to the production of H₂S during these reactions.

Fig. 2 Gas chromatograms of *n*-alkanes from the Ghareb Formation. *a*, Characteristic *n*-alkane distribution of the bituminous Ghareb Formation; *b*, *n*-alkane distribution of the lowermost bed. Separation on 6 foot $\frac{1}{8}$ inch column packed with 2% Apiezon L on Gas Chrom Z, programming 100–290 °C, 4 °C per min.



The process of single crystal calcite replacement of gypsum infillings of foraminiferal tests, thus envisaged, takes place in a combination of strictly defined conditions: water oversaturated with respect to calcium carbonate and gypsum, in the presence of organic material and sulphate reducing bacteria. It is probable that such replacement is generally indicative of this type of sedimentary environment.

BARUCH SPIRO
Zeev Aizenshtat,

Department of Geology,

The Energy Research Center,
The Hebrew University of Jerusalem, Israel

Received 13 June; accepted 5 July 1977.

- ¹ Reiss, Z. *Israel. Geol. Surv. Israel Bull.* 34, 1-23 (1962).
- ² Albrecht, P. & Ourisson, G. *Angew. Chem.* 10, 209-225 (1971).
- ³ Calvin, M. *Chemical Evolution* (Oxford University Press, Oxford, 1969).
- ⁴ Han, J. & Calvin, M. *Proc. natn. Acad. Sci. U.S.A.* 64, 436-443 (1969).
- ⁵ Han, J., McCarthy, E. D., Van Hoeben, W., Calvin, M. & Bradley, W. H. *Proc. natn. Acad. Sci. U.S.A.* 59, 29-37 (1968).
- ⁶ Winters, K., Parker, P. L. & Van Baalen, C. *Science* 158, 467-468 (1969).
- ⁷ Dembicki, H., Jr, Meinschein, W. G. & Hattin, D. E. *Geochim. cosmochim. Acta* 40, 203-208 (1976).
- ⁸ Welte, D. H. & Waples, D. *Naturwissenschaften* 60, 516-517 (1973).
- ⁹ Berner, R. A. *Principles of Chemical Sedimentology* (McGraw-Hill, New York, (1971)).

Inducer of pectic acid lyase in *Erwinia carotovora*

PECTIC acid (polygalacturonic acid) lyase (EC 4.2.2.2., PAL) formed in bacteria, catalyses the transesterificative cleavage of pectic acid¹. Because PAL both macerates plant tissues and causes cell leakage and death^{2,3}, it has often been investigated biochemically from the industrial and plant pathological point of view. Physiological studies, however, have been limited, probably because PAL is in part an extracellular enzyme⁴. Thus most studies of its inducibility have compared activities in filtrates of cells cultured on different carbon sources^{5,6}. But assay of an extracellular enzyme does not necessarily reveal changes in its rate of synthesis. In *Erwinia carotovora*, however, the PAL that can be purified from cell-free extracts is probably the same protein as the extracellular enzyme¹. In strain EC-1 of *E. carotovora*, non-induced PAL activity is three to four times greater in cell-free extracts than in the culture filtrate during log phase growth, and the relative rate of increase of intracellular enzyme activity after induction is 130 times greater than the rate of release of PAL into the culture filtrate (unpublished data). I therefore used this strain to study induction by measuring PAL activities in cell-free extracts. I report here that the inducer of PAL in *E. carotovora* may not be pectic acid itself but its breakdown product.

Maximum differential activity has been reported to be in the culture fluid grown on pectic acid⁶. To test this, after the addition of pectic acid into minimal medium of Mikula *et al.*⁷ (without sodium citrate) time course samples were taken, washed, sonicated and assayed by the thiobarbituric acid method⁸. The inducibility of PAL by pectic acid was shown by an increase in PAL specific activity (Fig. 1a). The long lag before the onset of induction, however, suggested that pectic acid was not inducing directly. It is unlikely that the lag simply reflects a low initial rate of uptake of pectic acid because (1) induction was further delayed and a growth lag was observed when pectic acid was added to washed cultures rather than directly to cultures grown in glycerol (Fig. 1a and b) or citrate (data not shown); (2) the lag for the induction by pectic acid was further delayed when 10^{-3} M EDTA was added in addition to pectic acid, and this could be abolished by addition of 10^{-3} M CaCl_2 in addition to EDTA and pectic acid (Fig. 1a). Because Ca^{2+} has been reported to activate many bacterial pectic

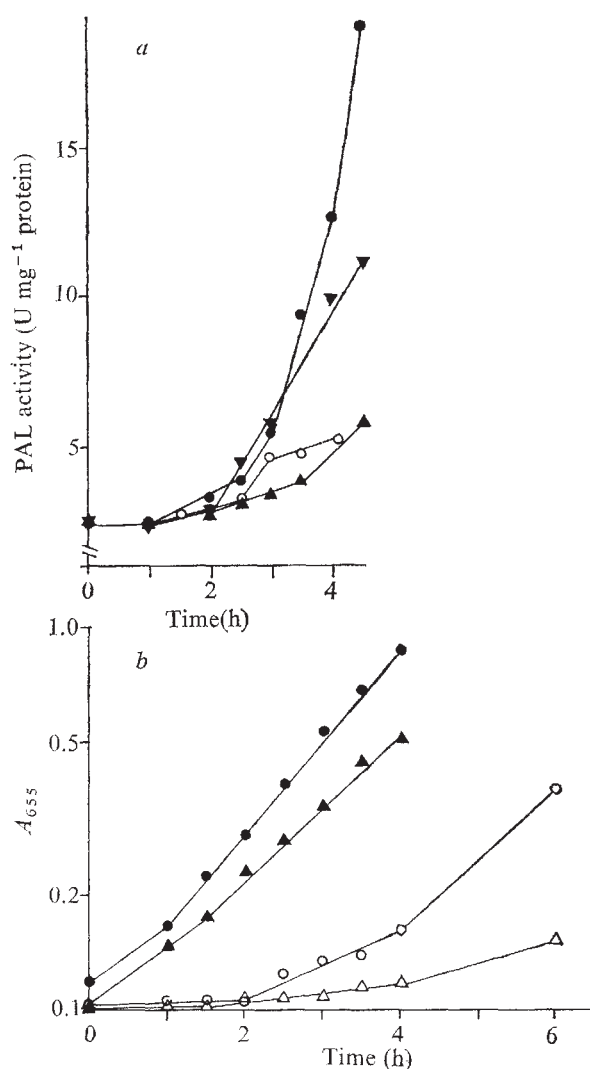


Fig. 1 Kinetics of the induction of PAL by pectic acid. *a*, Induction was initiated by the addition of 0.5% pectic acid, pH 8.3, with 10^{-3} M CaCl_2 or 10^{-3} M EDTA, either directly (closed symbols) or after washing (open symbols), to EC-1 cultures grown in minimal⁷ + 2% glycerol medium to A_{655} of about 0.1. At intervals, 6-ml samples were withdrawn, washed twice and resuspended in 1.5 ml of Tris-HCl buffer (0.05 M, pH 8.0). Suspensions were sonicated and the cell debris was removed by centrifugation for 15 min at 10,000g. PAL activity was measured by a modified thiobarbituric acid method⁸. One unit of PAL is defined as the amount of enzyme which produces 1 μmol of UDG per h. Specific activity is expressed as units of enzyme activity per mg of protein. Protein concentrations were determined by the method of Lowry¹⁴. *b*, During the induction experiment growth was measured as the increase in absorbance at 655 nm after the addition of pectic acid. ●, ○, Pectate; ▲, △, pectate + EDTA; ▼, pectate + EDTA + CaCl_2 .

acid lyases (including the *Erwinia* enzyme¹), these two observations suggest that some product of pectic acid degradation by the non-induced level of PAL in culture filtrate is the true inducing metabolite.

The true inducing metabolite of PAL was sought in time-course induction experiments after the addition of one of the purified metabolites of pectic acid into the minimal medium⁷ as a sole carbon source. The major end product of the cleavage of pectic acid by *E. carotovora* PAL is *O*-(4-deoxy- β -L-threo-hexo-pyranos-4-enyluronate)-(1-4)-D-galacturonic acid (unsaturated digalacturonic acid, UDG). The rapid induction was observed specifically when 2×10^{-3} M purified UDG⁹ was added (Fig. 2). Pectic acid is also degraded by hydrolytic polygalacturonase (EC 2.3.1.15., PG) of *E. carotovora*¹⁰. No induction was observed, however, after the addition of galacturonic acid,

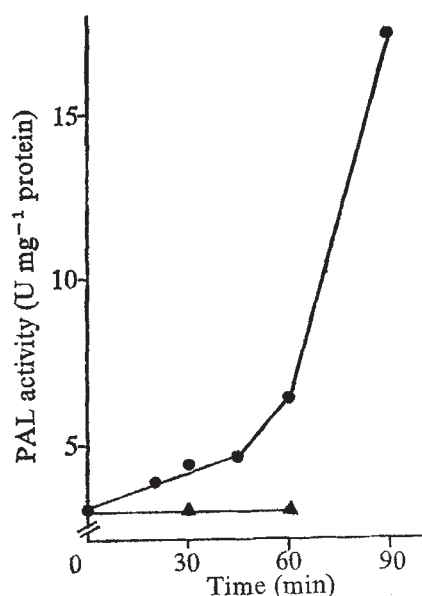


Fig. 2 Kinetics of the induction of PAL by UDG. The procedures were the same as in Fig. 1 except that 2×10^{-3} M UDG or 26×10^{-3} M galacturonic acid instead of pectic acid was added after washing the cultures. ●, UDG; ▲, galacturonate.

which is the major product of the cleavage of pectic acid by *E. carotovora* PG (Fig. 2)¹.

UDG is further metabolised to 4-deoxy-L-threo-5-hexoseulose uronic acid by oligogalacturonic acid lyase (EC 4.2.2.6, OGL)^{11,12}, but as the induction of PAL and OGL is uncoordinate in *Erwinia*¹², UDG rather than its further breakdown product is likely to be the true inducer. The inducer, however, might be a product of unsaturated digalacturonic acid formed by way of a minor pathway, as shown with *Escherichia coli* β -galactosidase, where the transfer products of lactose, 1,6-galactosido-glucose and galactosido-glycerol are inducers¹³.

Data concerning other plant pathogenic bacteria and evidence for catabolite repression of PAL will be published elsewhere. Product-induction may well be a mechanism for regulating other enzymes which have macromolecular substrates. The specific inhibition of PAL induction may also provide an approach to the control of plant diseases with non-toxic agents.

I thank Professors N. Okabe and M. Goto for encouragement, Dr C. Hatanaka for UDG, and Drs R. Bigelis, I. Tsujino, T. Mizuno and S. Nasuno for helpful discussions.

SHINJI TSUYUMU

Faculty of Agriculture,
Shizuoka University,
836 Ohya,
Shizuoka, Japan

Received 18 April; accepted 22 July 1977.

- Moran, F., Nasuno, S. & Starr, M. P. *Arch. Biochem. Biophys.* **123**, 298–306 (1968).
- Mount, M. S., Bateman, D. F., Basham, H. G. *Phytopathology* **60**, 924–931 (1970).
- Hall, J. A. & Wood, R. K. S. *Nature* **227**, 1226 (1970).
- Dean, M. & Wood, R. K. S. *Nature* **214**, 408–410 (1967).
- Zucker, M. & Hankin, L. J. *Bact.* **104**, 13–18 (1970).
- Macmillan, J. D. & Vaughn, R. H. *Biochemistry*, **3**, 564–572 (1964).
- Mikula, J. W., Stieglitz, B. I. & Calvo, J. M. *J. Bact.* **109**, 584–593 (1972).
- Weissbach, A. & Hurwitz, J. *J. biol. Chem.* **234**, 705–709 (1959).
- Okamoto, K., Hatanaka, C. & Ozawa, J. *Agric. biol. Chem.* **28**, 331–336 (1964).
- Goto, M. & Okabe, N. *Ann. Phytopathol. Soc. Jap.* **27**, 1–9 (1962).
- Preiss, J. and Ashwell, G. *J. biol. Chem.* **238**, 1571–1576 (1963).
- Hatanaka, C. & Ozawa, J. *Agric. biol. Chem.* **36**, 2307–2313 (1972).
- Platt, T., Müller-Hill, B. & Miller, J. H. in *Experiments in Molecular Genetics* (ed. Miller, J. H.) 358 (Cold Spring Harbor Laboratory, New York, 1972).
- Lowry, O. H., Rosebrough, N. J., Farr, A. J. & Randall, R. J. *J. biol. Chem.* **193**, 265–275 (1951).

Steroids stimulate secretion by insect Malpighian tubules

DURING the growth and development of insects there are rapid changes in the concentrations of the moulting hormones (ecdysteroids). In the locust *Locusta migratoria* the rate of excretion of the moulting hormones is one factor which regulates the ecdysteroid titre^{1,2}. The experiments reported here show that the presence of ecdysteroids causes an increase in the rate of urine production by the Malpighian tubules of the tsetse fly *Glossina morsitans*. Previously there has been no evidence to suggest that steroid hormones are involved in the control of excretion in insects³. These results may therefore indicate both a new function for steroids in insects and a new control system for insect excretory systems.

Teneral adult male flies, 24–48 h after emergence, were used in all experiments. Sections (about 1 cm) of their Malpighian tubules were removed and set up *in vitro* in drops of Ringer solution under liquid paraffin⁴. In the absence of a stimulant the tubules secreted very slowly but the addition of extracts of the thoracic ganglion of the fly (which contains the diuretic hormone⁵) or cyclic AMP caused a rapid increase in the rate of secretion. The high rate was maintained as long as the stimulants were present but rapidly reverted to the unstimulated level when they were removed (Fig. 1). The addition of ecdysone (α -ecdysone) or ecdysterone (β -ecdysone) to the bathing drop also caused an increase in the rate of secretion but the time course of this response was very different from that which was elicited by the diuretic hormone or cyclic AMP (Fig. 1). A delay of 20–40 min occurred between the addition of the ecdysteroid and the commencement of fluid secretion by the isolated Malpighian tubule. After the ecdysteroid was removed the rate of secretion returned to its unstimulated level only very slowly. Where tubules were left in bathing drops con-

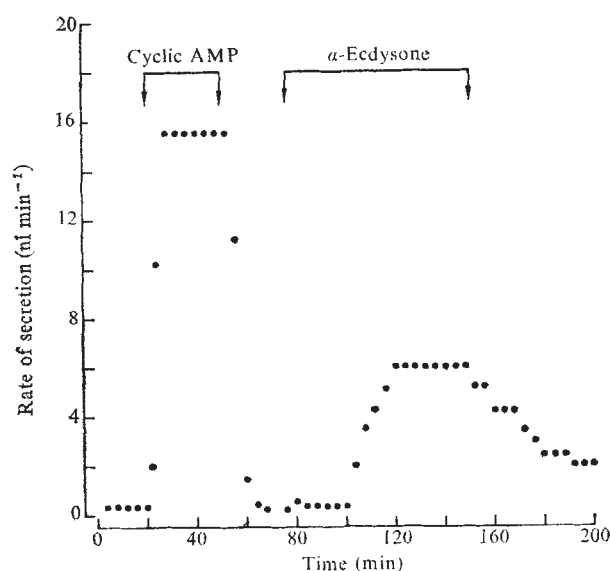


Fig. 1 Rate of secretion of a typical *in vitro* preparation of a *Glossina* Malpighian tubule. A tubule was isolated into a bathing drop of Ringer solution, composition (mM) NaCl, 120; KCl, 10; glucose, 20; malic acid, 3; citric acid, 2; $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 2; $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$, 1.5; $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$, 2; pH adjusted to 7 with NaOH and phenol red added to keep constant check on pH. The bathing drop was replaced by Ringer containing 10^{-3} M cyclic AMP for the period shown, after which the tubule was returned to basic Ringer before being transferred to Ringer containing 10^{-6} M ecdysone. Finally, the tubule was returned to basic Ringer. Experiments were performed at 25–28 °C.

taining the ecdysteroids they continued to secrete for many hours. At higher temperatures (28–31 °C), though initially the tubules secreted more rapidly in the presence of the ecdysteroids, the preparations deteriorated after 2–3 h and secretion ceased.

Ecdysone and ecdysterone elicited secretory responses of similar magnitudes. Expressed as a percentage of the maximum response of the tubule stimulated by cyclic AMP, 10^{-6} M ecdysone produced a response of $32.7 \pm 7.0\%$ (mean $\pm 2 \times \text{s.e.}$, $n = 8$) and 10^{-6} M ecdysterone a response of $26.3\% \pm 3.8\%$ ($n = 5$). Concentrations above 10^{-6} M were found to cause no further increase in the rate of secretion. But, the rate of secretion decreased with decreasing steroid concentrations below 10^{-6} M, down to a threshold response at 2×10^{-8} M. The response of the tubules at these low concentrations indicates that they would be stimulated to secrete by ecdysteroids at the concentration which is present in the haemolymph of teneral adult male *Glossina*. This was estimated by radioimmunoassay to be 375 pmol g^{-1} . Whole insect extracts of teneral male flies of the same age contained $203 \pm 38 \text{ pmol g}^{-1}$ (mean $\pm 2 \times \text{s.e.}$, $n = 12$; unpublished observations of D.L.W.). Approximately half the total ecdysteroid content of the fly is therefore present in the haemolymph. Whole fly extracts assayed by radioimmunoassay have shown a concentration of 600 pmol g^{-1} in adult male *Calliphora* (J.K. unpublished observations) and the concentration of ecdysterone in adult male *Drosophila* measured by gas-liquid chromatography (350 pmol g^{-1} fresh weight) is in fact higher than at any other stage of its life cycle⁶. It has been known for some time that ecdysteroids are present in adult insects⁷. In female *Locusta*⁸ and *Bombyx*⁹ they are present in the ovaries and oocytes but previously there has been no hormonal function attributed to the high concentrations of ecdysteroids in adult male insects.

The rates of secretion of Malpighian tubules in Ringer's solution alone, in Ringer containing ecdysteroids and in Ringer containing other biologically active steroids are compared in Table 1. In all cases a delay between the introduction of the steroid and the commencement of secretion occurred (range 20–80 min) and the return to the base rate of secretion was slow. The time course of the response by *Glossina* Malpighian tubules to these steroids seems similar both to that of larval *Drosophila* salivary glands, exposed to ecdysterone¹⁰ and to the response of vertebrate transporting epithelia to the mineralocorticoid aldosterone¹¹. It was thought that the delayed response of the *Glossina* tubule preparation might similarly be due to the time required for the sequence of events (receptor interaction, transcription and translation) necessary for cells to respond to a steroid hormone. The delayed recovery might reflect activity of the proteins mediating the action of the steroid hormone continuing after the stimulating steroid was removed. To verify this, Malpighian tubules were stimulated to secrete by ecdysone in the presence of $10 \mu\text{g ml}^{-1}$ actinomycin D which irreversibly inhibits transcription. Their response was not reduced (Table 1). If ecdysone stimulates secretion by *Glossina* Malpighian tubules by controlling protein synthesis it presumably therefore acts post-transcriptionally.

Another way in which steroids might increase the rate of secretion is by altering the permeability of the basal membrane of the tubule cells. The rate of secretion of *Glossina* Malpighian tubules is thought to be determined by the permeability of their basal membrane to sodium¹² and it has been suggested that ecdysteroids are able to alter the permeability of cell and nuclear membranes to sodium and potassium¹³. Cholesterol is also known to alter the permeability of cell membranes¹⁴.

The results of these experiments suggest new lines of investigation into both the control of insect Malpighian

Table 1 Rate of secretion of isolated Malpighian tubules in the presence of steroids

Steroid present	Mean rate of secretion (nl min ⁻¹)	2 × s.e.	n
None	0.3	0.09	12
Ecdysone	4.2	0.98	20
Ecdysterone	3.4	1.06	9
Cortisol	3.1	1.16	7
Cholesterol	3.3	1.88	6
Aldosterone	4.5	1.13	9
Ecdysone + $10 \mu\text{g ml}^{-1}$ actinomycin D	5.1	1.22	10

Steroids were used at a concentration of 10^{-6} M, except for cholesterol which is only slightly soluble in water. A saturated solution of cholesterol in Ringer (containing approximately 0.2 mg per 100 ml H₂O) was centrifuged before it was added to the isolated preparation. The concentration of cholesterol in solution was approximately 5.17×10^{-6} M.

tubules and the function of ecdysteroids in insects. It has subsequently been shown that in *Locusta* also ecdysterone and cholesterol stimulate secretion by *in vitro* preparations of the Malpighian tubules (A. Bernstein and W. Mordue, in preparation). *Glossina* tubules respond to ecdysteroids at concentrations within the physiological range and this response suggests a function for the high concentrations of ecdysteroids present in adult male insects. The action of ecdysteroids (which control the growth and development of the larval stages of the insect) on the process of excretion in the adult insect is especially interesting since the Malpighian tubules are one of the few organs which remain intact throughout metamorphosis. We have little knowledge of the identities or titres of steroids circulating in the haemolymph of adult insects. But, as this investigation suggests that they may have regulatory functions in insects, over and above that of controlling the development of the larval and pupal stages, this may become an interesting new field of study.

We thank Dr J. D. O'Connor and Professor A. E. Kellie for advice about preparation of anti-sera for the radioimmunoassay of ecdysteroids. J. K. thanks the British Council for a grant to visit Britain and J. D. G. and D. L. W. thank the Ministry of Overseas Development for support.

JULIAN D. GEE*

DAVID L. WHITEHEAD

Tsetse Research Laboratory,
University of Bristol, School of Veterinary Science,
Langford, Bristol, UK

JAN KOOLMAN

Physiologisch-Chemisches Institut,
Phillips-Universität Marburg,
D-3550 Marburg, West Germany

Received 25 April; accepted 26 July 1977.

*Present address: Parasitology Department, Central Research, Pfizer Limited, Sandwich, UK.

- Koolman, J., Hoffman, J. A. & Dreyer, M. *Experientia* 31, 247–249 (1975).
- Hoffman, J. A. & Koolman, J. *J. Insect Physiol.* 20, 1593–1601 (1974).
- Gee, J. D. in *Transport of Ions and Water in Animals* (eds Gupta, B. L., Moreton, R. B., Oschman, J. L. & Wall, B. J.) (Academic, New York and London, 1977).
- Gee, J. D. *J. exp. Biol.* 64, 357–368 (1976).
- Gee, J. D. *J. exp. Biol.* 63, 391–401 (1975).
- Ginsburg, D. thesis, Univ. York (1977).
- Karlson, P. & Stamm, D. *Hoppe-Seyler's Z-physiol. Chem.* 306, 109–111 (1956).
- Lagueux, M., Hirn, M. & Hoffman, J. A. *J. Insect Physiol.* 23, 109–119 (1977).
- Legay, J.-M., Calvez, B., Hirn, M. & De Reggi, M. L. *Nature* 262, 489–490 (1976).
- Ashburner, M. & Richards, G. in *Insect Development* (ed. Lawrence, P. A.) 203–225 (Blackwell, Oxford, 1976).
- Edelman, I. S. in *Transport Mechanisms in Epithelia, Alfred Benzon Symposium* 5, (eds Ussing, H. H. & Thorn, N.) 185–204 (Munksgaard, Copenhagen, 1973).
- Gee, J. D. *J. exp. Biol.* 65, 323–332 (1976).
- Kroeger, H. in *Metamorphosis* (eds Etkin, W. & Gilbert, L. I.) 185–219 (North-Holland, Amsterdam, 1968).
- Szabo, G. *Nature* 252, 47–49 (1974).

Meiotic crossing-over in lily and mouse

CYTOLOGICAL descriptions of meiosis have long been a staple of biology textbooks, but a biochemical account of the process with a comparable claim to universality has been unavailable. We present here evidence for a common pattern of DNA metabolism in male meiotic cells of lilies and rodents during the zygotene-pachytene stages when crossing-over is presumed to occur. The prevalence of a common pattern between species that are as phylogenetically distant as lily and mouse has led us to conclude that the organisation for meiotic crossing-over in at least higher eukaryotes is probably universal in distribution. Four components of the pattern have been identified in separate studies of lilies, mice and rats, most of which have been or are being published elsewhere¹⁻³: the transient appearance of a protein ('R-protein') that facilitates DNA reannealing; the programmed introduction of single strand nicks on completion or near completion of chromosome pairing; the repair of endogenously formed nicks and gaps either immediately following or overlapping the interval of nicking; the preferential localisation of gaps and nicks in specific DNA sequences.

We described previously a protein in nuclei of *Lilium* meiocytes but not of its somatic cells which, like the gene-32 protein, facilitated DNA reannealing⁴. A preliminary report on the presence of a similar protein in testicular nuclei of various mammals was also published⁵. This protein was judged to be significant to chromosome pairing and crossing-over because of its absence during premeiotic chromosome replication, its prominence during zygotene and pachytene, and the reduction of pairing and chiasma formation on disrupting its normal association with nuclear lipoprotein by colchicine treatment⁶. A more detailed study⁷ of the R-protein purified from rat testicular nuclei showed it to have properties distinctly different from those of unwinding proteins in calf thymus which have been thoroughly analysed by Herrick and Alberts⁸. The R-proteins from rat and lily, on the other hand, are very similar to the T4 gene-32 protein with respect to promotion of DNA reassociation and of duplex unwinding in Mg^{2+} -free medium. They are distinctive, however, in that the capacity for reassociation and DNA binding can be destroyed by phosphorylation with a cyclic AMP-dependent protein kinase. Dephosphorylation with alkaline phosphatase restores the binding capacity⁷, but, as we have since observed, not the capacity for reassociation.

We fractionated mouse spermatocytes according to spermatogenic stage and analysed each of the fractions for R-protein activity. Although the different meiotic stages of spermatocytes cannot be separated as well as those of *Lilium* microsporocytes, the respective profiles of R-protein activity during meiosis are very similar (Fig. 1). In both organisms, the R-protein is most prominent during the intervals of zygotene and pachytene when pairing and crossing-over occur. In neither organism is there any evidence for a substantial presence during premeiotic DNA replication when DNA synthesis is at least 300 times greater than during meiotic prophase. Circumstantial evidence thus indicates that in the case of the R-protein, unlike the gene-32 or similar microbial proteins, the recombinational function has been separated from the replicative one. Although the precise role of the R-protein in meiosis remains to be defined and its apparently marginal presence in somatic tissues needs to be explored, the evidence now available points to the conclusion that the R-protein is essential to chromosome pairing and/or crossing-over among higher eukaryotic organisms.

In *Lilium*, single strand nicks and gaps appear in nuclear

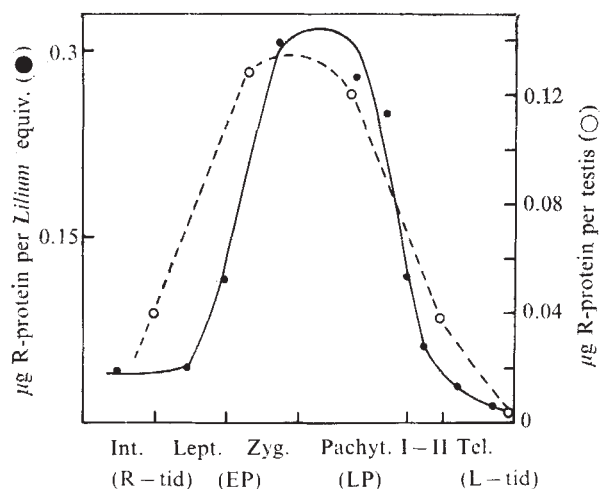


Fig. 1 Changes in R-protein during meiosis in lily microsporocytes (●) and rat spermatocytes (○). The data on lily are from previous studies⁴. To permit a rough comparison in R-protein content between the two organisms, we have recalculated the values on lilies so that the numbers for lily and mouse reflect approximately equal amounts of meiocyte DNA. Spermatocytes were separated by a modified Staput procedure⁹ into fractions designated by the predominant cell type: round spermatid (R-tid), early prophase (EP), late prophase (LP), and late spermatid (L-tid). Although R-tids are postmeiotic, the fraction is the only one containing cells in premeiotic S-phase and very early prophase. Separation of meiotic stages in the lily is unambiguous: Premeiotic interphase including S-phase (int.), leptotene (lept.), zygotene (zyg.), pachytene (pachyt.), Divisions I and II (I-II), telophase (tel.). Nuclei from pooled spermatocytes were suspended in a buffered medium consisting of 20mM Tris-HCl, pH 7.5–25mM KCl–3mM $CaCl_2$ –0.1mM dithiothreitol, sonicated for 45 s at 0°C, and the chromatin-free lipoprotein fraction isolated^{8,11}. The lipoprotein material was resuspended in buffered medium and assayed for duplex and single-stranded DNA binding activity¹¹. A discontinuous sucrose gradient was used to recover the bound DNA. The difference between the amounts of single strand and duplex DNA bound was considered to be R-protein. The activities are expressed in terms of R-protein content, based on assays of a purified protein which bound approximately 0.09 µg (³H)T7-DNA per µg R-protein.

DNA at about the time that the pairing of homologous chromosomes is completed⁹. We have interpreted such endogenously regulated nicking as a necessary condition for crossing-over and have adduced evidence for the absence of nicking in situations where crossing-over fails to occur. A pattern of DNA nicking similar to the one in lilies has now been found in mouse spermatocytes³. In both species, zonal sedimentation profiles of single stranded DNA are bimodal at pachytene and unimodal at all other stages of meiosis, thus reflecting the introduction of nicks at the pachytene stage. The unimodal profiles peak at about 105S in the lily and 160S in the mouse, corresponding to molecular weights of 1.8 and 5.1×10^6 (ref. 10). Significantly perhaps, the slower sedimenting component in each of the bimodal profiles peaks at about 62S corresponding to a molecular weight of 48×10^6 . The changes in proportion of 62S DNA at different stages of meiosis in lily and mouse are shown in Fig. 2. Although the fraction of 62S DNA at pachytene is slightly greater in the mouse, the total number of nicks per meiocyte is much larger in lilies, which have a haploid DNA content about 20 times that of mice. Since there are approximately 36 chiasmata per lily microsporocyte and 20 per mouse spermatocyte, the efficiency of converting nicks into crossovers is greater in mouse than in lily. There may indeed be an inverse relationship between genome size and efficiency of such conversion. In lily, there are about 1×10^4 nicks per crossover; in yeast, where a pattern of nicking similar to the one in lily has been

observed. 50% of the nicks seem to be converted into reciprocal crossovers¹¹. Although we have no information on the mechanism relating nicking to the ultimate event of crossing-over, the occurrence of meiotic nicking in yeast lily and mouse, is compelling evidence for the universality of endogenously regulated nicking in eukaryotes as a background condition for crossing-over.

Implicit in the profiles shown in Fig. 2 is the occurrence of sufficient repair synthesis during pachytene to restore the linear integrity of the DNA to its prepachytene level. Direct evidence for repair replication has been obtained in both mouse and lily by pulse labelling of pachytene cells that had previously incorporated bromodeoxyuridine during the premeiotic S-phase^{3,9}. But, meiotic cells, like somatic ones, can repair DNA lesions at almost any stage of development, so that nicking, whether endogenous or exogenous in origin, would be expected to promote repair synthesis. That endogenous pachytene repair synthesis shares many features with exogenously induced repair has been adequately documented. There are, nevertheless, two distinguishing features of the endogenous process. One of these is the elevated capacity for repair in pachytene cells, a property that has been well demonstrated in the mouse¹², and can also be demonstrated in lily. The other, more significant feature, concerns specific sites of nicking and repair, as discussed below.

Commonly, frequency of crossing-over between two loci in a chromosome is considered to be a function of the physical distance between them. Although broad exceptions to this relationship have been documented¹³, it might, nevertheless, be supposed that endogenous meiotic repair synthesis would be randomly distributed in the genome. This, however, is not the case. *C₀t* profiles of DNA synthesised during pachytene show that the endogenous synthesis occurs preferentially in moderately repeated sequences (Fig. 3). Such preference is a unique characteristic of meiotic repair and not of repair in general; radiation-induced repair tracks the *C₀t* profile of total DNA regardless of meiotic stage. In lily, 50%–70% of endogenous pachytene repair occurs in sequences that are repeated about 1–2,000 times per genome and constitute at least 0.1% of

Fig. 2 Changes in sedimentation behaviour of single-stranded DNA prepared from microsporocytes and spermatocytes at different stages of meiosis. The profiles are constructed from data obtained in analyses of gently lysed preparations over alkaline glycerol gradients. The proportion of 62S DNA is calculated from the weight distribution of DNA in the region of the gradient containing the 62S component. Designation of meiotic stages is the same as in Fig. 1. Stages beyond pachytene have been grouped under post-pachytene (Post-pachy.). Assignment of sedimentation characteristics to specific spermatocyte stages is easier than in the case of the R-protein because the 62S component is prominent only during the pachytene stage. ●, Lily; ○, mouse.

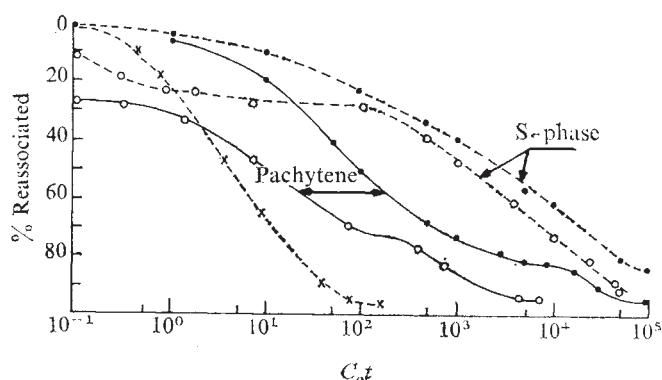
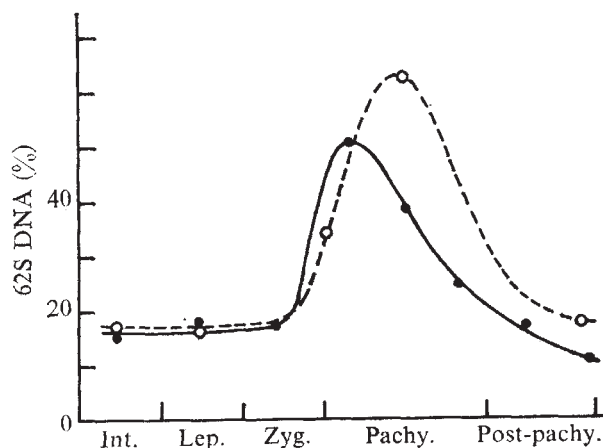


Fig. 3 Reassociation characteristics of mouse and lily DNA synthesised during the pachytene stage. The data displayed are from earlier analyses of *Lilium* microsporocytes¹⁸ and from recent analyses of mouse spermatocytes⁷. In the case of the mouse, the data on pachytene are from mice treated with hydroxyurea to inhibit semiconservative synthesis. In the absence of hydroxyurea, the pachytene profile, though essentially the same, is less pronounced because of contamination by S-phase cells⁶. *C₀t* characteristics of S-phase labelled DNA are included for comparison. ●, Lily; ○, mouse; ×, *Escherichia coli*.

the total DNA¹⁴. The precise lengths of the repeats have not been determined, but we estimate them to be in the neighbourhood of 100–200 bases¹⁵. In mouse, 50% of the repair occurs in sequences that are repeated about 400 times per genome; the proportion of DNA thus involved is undetermined but it cannot be detected by absorbance measurements, and is probably less than 1–2%. There is, however, one feature of pachytene synthesis in mouse spermatocytes which is not evident in lilies. In the profile shown in Fig. 3, about 25% of the pachytene label reassociates at a *C₀t* value below 10^{-2} . This rapidly reannealing region is being analysed in greater detail, but it is already clear that with 400–500 nucleotide fragments about 15% of the total pachytene label reassociates as foldback DNA, and that 1/3 of the label present in the foldbacks is in duplex form. This observation is pertinent to speculations about the involvement of hairpin loops in crossing-over^{16,17}. The location of repeated sequences in lily and mouse in relation to single-copy ones needs to be determined, but it is evident from Fig. 3 that pachytene labelling does not occur exclusively in repeat sequences but is also present among the lower repeat sequences.

The evidence, limited though it is, points unambiguously to the conclusion that in organisms as diverse as lily and mouse, metabolic events that are temporally associated with meiotic crossing-over share a common pattern. The preferential and programmed nicking of DNA in regions of moderate repeats, the similarity in spacing between nicked sites, and the temporal correlation between endogenous repair synthesis and cytological stage, are impressive examples of a conserved biochemical program for meiosis. In our opinion, these examples provide for the observed regularity in crossing-over among higher organisms.

This work was supported by grants from the USNIH (HD 03015) and the NSF (PCM 76-09660).

YASUO HOTTA
ANN C. CHANDLEY*
HERBERT STERN

Department of Biology
University of California, San Diego
La Jolla, California 92093

Received 21 April; accepted 26 July 1977.

*Permanent address: MRC Clinical and Population Cytogenetics Unit, Western General Hospital, Crewe Road, Edinburgh, UK.

- ¹ Stern, H. & Hotta, Y. *Phil. Trans. R. Soc. Lond. B* 277, 277–293 (1977).
- ² Chandley, A. C., Hotta, Y. & Stern, H. *Chromosoma* 62, 243–253 (1977).
- ³ Hotta, Y., Chandley, A. C. & Stern, H. *Chromosoma* 62, 255–268 (1977).
- ⁴ Hotta, Y. & Stern, H. *Dev Biol.* 26, 87–99 (1971).
- ⁵ Hotta, Y. & Stern, H. *Nature new Biol.* 234, 83–86 (1971).
- ⁶ Hotta, Y. & Shepard, J. *Molec. gen. Genet.* 122, 243–260 (1973).
- ⁷ Mather, J. & Hotta, Y. *Exp Cell Res.* (in the press).
- ⁸ Herrick, G. & Alberts, B. J. *biol. Chem.* 251, 2124–2132 (1976).
- ⁹ Hotta, Y. & Stern, H. *Chromosoma* 46, 279–296 (1974).
- ¹⁰ Studier, F. W. *J. molec. Biol.* 11, 373–390 (1965).
- ¹¹ Jacobson, G. K., Piñon, R., Esposito, R. E. & Esposito, M. S. *Proc. natn. Acad. Sci. U.S.A.* 72, 1887–1891 (1975).
- ¹² Kofman-Alfaro, S. & Chandley, A. C. *Exp Cell Res.* 69, 33–44 (1971).
- ¹³ Jones, G. H. *Heredity* 32, 375–387 (1974).
- ¹⁴ Smyth, D. R. & Stern, H. *Nature new Biol.* 245, 94–96 (1973).
- ¹⁵ Hotta, Y. & Stern, H. in *The Eukaryote Chromosome* (eds Peacock, W. J. & Brock, R. D.) 283–300 (Australian National University Press, Canberra, 1975).
- ¹⁶ Sobell, H. M. *Proc. natn. Acad. Sci. U.S.A.* 72, 279–283 (1975).
- ¹⁷ Wagner, Jr, R. E. & Radman, M. *Proc. natn. Acad. Sci. U.S.A.* 72, 3619–3622 (1975).

Evidence for two active X chromosomes in germ cells of female before meiotic entry

THERE is considerable evidence that, as a result of some early embryonic event, the genes of one X chromosome in each somatic cell of the human female are not expressed¹. Studies of somatic tissues and cultured cells (including fibroblast clones) from human embryos, heterozygous for the common electrophoretic variant of glucose-6-phosphate dehydrogenase (G6PD), indicate that a single active X is present in cells from various tissues, at least by 5 weeks from conception². On the other hand, the presence of the heteropolymorphic form of the enzyme in oocytes of the heterozygous adult female³ and a 16-week-old foetus⁴ is compelling evidence that, in meiotic stage germ cells, there are two active X chromosomes; however, the basis for the two active chromosomes in oocytes is not clear. It is conceivable that germ cell progenitors escape inactivation because inactivation occurs when cells destined to become germ cells have already been imprinted. Alternatively, only a single X chromosome may be active in all cells of the early zygote, with the second X activated only in germ cells at some time after their differentiation. The third possibility, that germ cells are subject to inactivation, but that reactivation occurs when meiosis commences, has been suggested by Gartler *et al.* because they did not observe the heteropolymer in ovaries from a 12-week-old human foetus heterozygous for the G6PD variant^{5,6}. The ovary at that developmental stage contains approximately 23% germ cells, with a preponderance of premeiotic and leptotene germ cells⁷. But the implication that two functional X chromosomes are required for the onset of mammalian meiosis is not supported by evidence that XO mice produce normal gametes⁸. Using human foetal material, we have now obtained evidence against the third possibility.

We have studied ovarian extracts from foetuses heterozygous for the G6PD_A variant. Specimens were obtained

(according to an approved protocol and with maternal consent) from dead foetuses in the Fertility Control Unit of the Johns Hopkins Hospital, where every effort is made to assure the accuracy of historical data. Electrophoresis for G6PD was carried out on cellulose acetate gels as before². During this study, we obtained G6PD isozyme patterns from 35 males and 49 females of foetal age 14–21 weeks. The patterns observed in lung tissue from these specimens indicate that the frequencies of the G6PD_A and G6PD_B alleles in the foetus are essentially that observed in somatic cells of a comparable adult population, and that, as expected, males have a single isozyme (Table 1). The age of each specimen was estimated from time of conception, on the basis of last menstrual period and measurements of mean foot length⁹ and crown–rump length of the foetus (Table 2). From specimens obtained after urea–prostaglandin infusion, we ascertained 17 females of 14–21 weeks gestation who were heterozygous for the G6PD variants, and obtained ovarian tissue from 10 of them. From examination of more than 100 specimens obtained from suction procedures, we obtained a single appropriate specimen of ovary from a foetus of 8 weeks'

Table 1 G6PD phenotype of foetal lung specimens

	Male	Female
No. of foetal specimens	35*	49
G6PD _{AB}	0	17
G6PD _A	7	6
G6PD _B	28	26

* 23 further male specimens were ascertained, but not analysed for G6PD.

conceptual age. Foetal specimens were assayed directly for the G6PD phenotype and, in each case, samples of lung were used as the somatic cell control for the ovarian specimen. Ovarian extracts were enriched for germ cells by pressing the foetal ovaries gently under a cover glass^{5,10}.

The results of electrophoretic studies of these 11 specimens are presented in Table 2 and Fig. 1. In each case, lung specimens revealed the heterozygosity, having both A and B isozymes. In all ovarian specimens studied, there were three isozymes with the heteropolymer migrating between the A and B bands. The intensity of the heteropolymer varied considerably. The band ratio of 1A:2 heteropolymer:1B, which might be expected for the three enzymes in a pure population of oocytes, was not seen even in the specimens from the most mature foetuses (20–21 weeks), indicating dilution of the ovarian specimen by non-germ cells. We confirmed previous observations that the heteropolymer was less intense in cells of younger

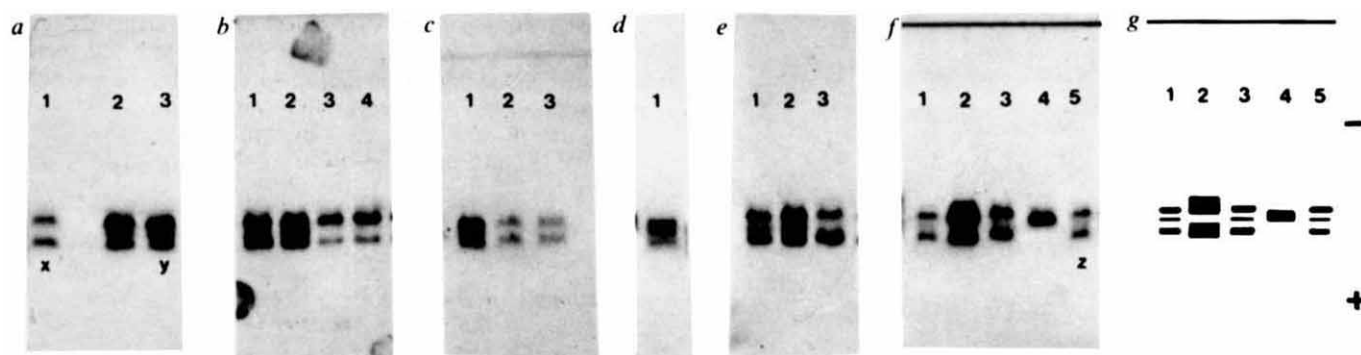


Fig. 1 G6PD isozyme patterns from foetal somatic and germ cell extracts. a, M-63, 19–20 weeks: 1, lung; 2, ovary; 3, ovary enriched for germ cells. b, M-82, 17 weeks: 1, ovary; 2, ovary enriched for germ cells; 3 and 4, lung. c, M-46, 17 weeks: 1, ovary enriched for germ cells; 2 and 3, lung. d, M-41, 16–17 weeks: 1, ovary enriched for germ cells. e, M-73, 14–15 weeks: 1, ovary; 2, ovary enriched for germ cells; 3, lung. f and g, S-19, 8 weeks: 1 and 5, ovary enriched for germ cells; 2, lung; 3, ovary; 4, G6PD_B marker. x, y and z indicate the source of the tracing in Fig. 2.

Table 2 Characteristics of foetal specimens heterozygous for the G6PD variants where ovaries were analysed

Specimen	Foetal age in weeks				G6PD phenotype	
	From last menstrual period	Estimated menstrual*	Estimated gestational†	Probable foetal age	Lung	Ovary
M49	24	22-23	21	21	==	==
M43	18-19	22-23	20	20	==	==
M63	13-17	21-22	19	19-20	==	==
M53	21	20-21	19	19	==	==
M82	22	18	17	17	==	==
M115	20-21	18	17	17	==	==
M46	19	18	17	17	==	==
M41	20	19	16	16-17	==	==
M28	16	—	16	15-16	==	==
M73	14	16	15	14-15	==	==
S19	9-10	9-10	8	8	==	==

* Based on mean foot length.

† Based on crown-rump length.

specimens³, most likely because of relatively greater contamination of these specimens by ovarian somatic cells. This is consistent with the lesser number of germ cells known to be in ovaries of younger fetuses⁷⁻¹¹. In contrast to previous observations³, we observed a heteropolymer even in the specimen from the 8-week-old foetus (Figs 1f and g and 2), at a time when the germ cell population is essentially premeiotic⁷⁻¹¹. Although a few germ cells in the leptotene and zygotene stages of first meiotic metaphase have been described in the 12-13-week ovary, meiotic cells are not found in significant numbers until 16 weeks from conception¹². The ovary of the 8-week specimen was at the lower limit of size necessary for the identification of the pertinent tissue; therefore, it is unlikely that studies of isozyme analysis of ovaries in younger foetal specimens are feasible.

Our observations indicate that two X chromosomes are functioning in female germ cells, even before meiotic entry, and they are supported by the cytological observations of Ohno, who did not observe a precociously condensed X chromosome in mouse oogonia, nor in cells entering meiosis¹². Because there seemed to be one condensed X chromosome in primordial germ cells of the yolk sac, he suggested that germ cells are X-inactivated but reactivated at the time of differentiation of primordial

germ cells into oogonia in the ovary. The presence of two active X chromosomes in premeiotic germ cells, reported here, does not differentiate between escape from inactivation and reactivation (or activation of the second X), but excludes reactivation coincident with the onset of meiosis.

This work was supported by a grant from NIH.

BARBARA R. MIGEON
KAREN JELALIAN

Department of Pediatrics,
Johns Hopkins University School of Medicine,
Baltimore, Maryland 21205

Received 11 May; accepted 20 June 1977.

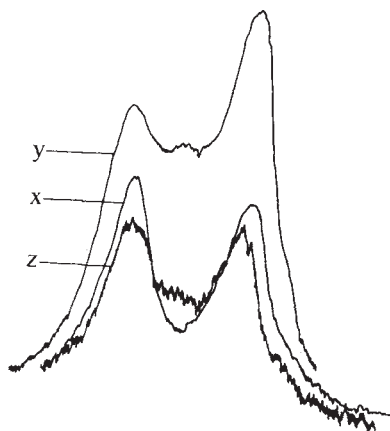
- 1 Lyon, M. F. *Biol. Rev.* 47, 1-35 (1972).
- 2 Migeon, B. R. & Kennedy, J. P. *Am. J. hum. Genet.* 27, 233-239 (1975).
- 3 Gartler, S. M., Liskay, R. M., Campbell, B. K., Sparkes, R. & Gant, N. *Cell Differentiation* 1, 215-218 (1972).
- 4 Gartler, S. M., Liskay, R. M. & Gant, N. *Expl Cell Res.* 82, 464-466 (1973).
- 5 Gartler, S. M., Andina, R. & Gant, N. *Expl Cell Res.* 91, 454-457 (1975).
- 6 Gartler, S. M. & Andina, R. *J. Adv. hum. Genet.* 7, 99-140 (1976).
- 7 Baker, T. G. *Proc. R. Soc., Lond. B* 158, 417-433 (1963).
- 8 Cattanaach, B. M. *Genet. Res.* 3, 487-490 (1962).
- 9 Streeter, G. L. *Carnegie Inst. Contrib. Embryol.* 11, 145-170 (1921).
- 10 Blandau, R. J., White, B. J. & Rumby, R. E. *Fertil. Steril.* 14, 482-489 (1963).
- 11 Ohno, S., Klinger, H. P. & Atkins, N. B. *Cytogenetics* 1, 42-51 (1962).
- 12 Ohno, S. in *Second int. Conf. Congenital Malformations*, 36-40 (National Foundation, New York, 1963).

Transcriptional controls and dosage compensation in *Drosophila melanogaster*

DOSAGE compensation is the equalisation of X-linked gene products in males and females. In *Drosophila* there is substantial evidence to support the contention that both X chromosomes are simultaneously active in somatic cells of females¹, and that the regulatory mechanism operates at the level of transcription^{2,3}. Most discussions of this mechanism have focused on the modulation of regulatory factors controlling the rate of RNA synthesis per template^{4,5}. A change in the level of transcription may also be achieved by altering the quantity of template. We present here various arguments and some experimental evidence discounting controlled variation of total template as the basis for dosage compensation in *Drosophila melanogaster*.

Three means by which the number of copies of X-linked genes may be equalised in male and female nuclei come to mind. First, the X chromosome may replicate earlier during the phase in males than in females. This is thought to be the case in polytenic nuclei of larval salivary glands^{6,7}. During a portion of the cell cycle, old and new copies of X-linked genes would be available

Fig. 2 Tracing from Joyce-Loebl recording microdensitometer showing G6PD_{AB} isozyme pattern x without heteropolymer from lung shown in Fig. 1a, slot 1; y with prominent heteropolymer from ovary shown in Fig. 1a, slot 3; and z with heteropolymer from ovary of 8-week specimen shown in Fig. 1f, slot 5.



for transcription in male cells; the number of genes per cell would, therefore, be the same in males and females until that time when replication of X-linked genes occurred in the latter. A serious objection to this model is based on the observation that the S phase constitutes at most 30%–40% of the cell cycle (measurements performed on cleaving embryos and cells in culture)^{8–10}. The male cells, therefore, spend a large portion of their existence in G1 or G2 where the dosage of their X-linked genes relative to that of autosomal genes is either 1:2 or 2:4. Furthermore, if the difference in time of X chromosome replication between males and females were significant, a considerable proportion of the cells sampled at any given time in males and in females should have equivalent X DNA content. But, microspectrophotometric measurements of Feulgen-stained chromosomes indicate that the paired X's in the salivary gland nuclei of female larvae have twice the DNA content of the single X in males (each measurement is made in reference to an autosomal, internal control)^{11–12}. It is, therefore, improbable that the early replication of X-linked genes in male cells could lead to equal transcriptional steady states in males and females.

A second possibility for template modulation would be the differential replication of X-linked genes in males. The unequal replication of specific sequences of the polytenic X chromosome in larval salivary glands is well documented. In a fully mature salivary gland chromosome, the centric heterochromatin including the highly repetitive DNA sequences has not undergone any endoreplication; the nucleolus organiser region containing the ribosomal RNA cistrons is present in 150 to 200 laterally redundant copies; the euchromatic portion of the chromosome is represented by more than 1,000 copies^{13,14}. If the coding segments of all structural genes were to undergo an extra round of replication in male cells, their dosage would become equal to that of female cells. Recent experimental evidence makes the differential replication model of dosage compensation unlikely. Electron microscope measurements of nascent RNA transcripts suggest that these molecules are of considerable size, perhaps corresponding to entire chromomeres which, in *Drosophila*, average 30 kilobases (kb) in length¹⁵. The median size of poly(A)-containing nuclear RNA is 6 kb, representing 20% of an average chromomere¹⁶. In terms of the model, this should lead to a substantial amount of extra DNA in males which, as mentioned above, does not seem to be present.

Finally, the equalisation of effective X chromosome

coding sequences in males and females may be achieved through polyteny itself. If only a small fraction of all strands were active in transcription, males may compensate for X-linked gene dosage differences by transcribing twice the number of strands per X chromosome as would be active in females. Implicit to this model is the contention that when gene activity is measured in whole organisms at the level of enzyme activity or of a terminal phenotypic product (eye pigment, for example), the compensatory effect stems from gene activity in polytenic cells. This cell type is found in both larvae and adults and may be ubiquitous enough to mask the lack of compensation in the strictly diploid tissues of the organism.

To test whether dosage compensation is the result of some mechanism inherent to polytenisation, we have compared the expression of X-linked genes in imaginal disks and larval brain to that in larval fat body. The brain is mostly diploid with a very small fraction of cells which may be polytenic^{17,18}; imaginal disk cells are diploid¹⁷; fat body cells are clearly polytenic¹⁸. Table 1 shows activity levels of two X-linked enzymes (glucose-6-phosphate dehydrogenase—G6PD, and 6-phosphogluconate dehydrogenase—6PGD) normalised to the activity levels of three autosomal enzymes (NADP-dependent isocitrate dehydrogenase, α -glycerophosphate dehydrogenase, and aldehyde oxidase). Measurements were performed in larval tissues and adult thoraces and heads. All male individuals carried w^+Y , a Y chromosome bearing a small X fragment which includes the structural gene of 6PGD. Gene dosage for this enzyme is, therefore, the same in males and females, providing control evidence that activity differences of the magnitude expected could be detected by our experimental procedure. The male–female ratios of normalised G6PD activity are equal to or slightly greater than one, while the ratios of 6PGD activity are slightly less than or equal to two, in disks, fat body and adult tissues. These observations suggest the occurrence of dosage compensation in all these tissues.

In summary, three possible models for equalising the number of copies of X-linked genes in *Drosophila* males and females have been considered. Existing data regarding the amount of DNA in X chromosomes and the proportion of the S phase in the cell cycle have been used to discredit early replication of the male X as a possible explanation for dosage compensation. It is unlikely that differential endoreplication of X-linked structural genes is occurring in males. Finally, we have shown that compensation cannot be ascribed to polytenisation *per se*. Therefore, we conclude that mechanisms that vary the relative

Table 1 Normalised X-linked gene activities in males and females

		G6PD/IDH	6PGD/IDH	G6PD/AO	6PGD/AO	G6PD/GPDH	6PGD/GPDH
♂	Disk	0.316 ± 0.03 (5)	0.698 ± 0.039 (5)	0.265 ± 0.054 (5)	0.575 ± 0.094 (5)		
♀	Disk	0.291 ± 0.022 (5)	0.381 ± 0.03 (5)	0.267 ± 0.032 (5)	0.345 ± 0.05 (5)		
♂/♀	Disk	1.08	1.83	0.99	1.67		
♂	Fat body	0.97 ± 0.08 (6)	2.31 ± 0.11 (6)			1.11 ± 0.1 (3)	2.57 ± 0.09 (3)
♀	Fat body	0.79 ± 0.06 (6)	1.11 ± 0.11 (6)			1.09 ± 0.12 (3)	1.57 ± 0.18 (3)
♂/♀	Fat body	1.22	2.08			1.01	1.64
♂	Adult	0.301 ± 0.02 (7)	0.416 ± 0.016 (7)	0.495 ± 0.012 (3)	0.545 ± 0.016 (3)		
♀	Adult	0.324 ± 0.031 (7)	0.254 ± 0.019 (7)	0.410 ± 0.061 (3)	0.296 ± 0.039 (3)		
♂/♀	Adult	0.93	1.64	1.21	1.84		

♂ Signs for male; ♀ signs for female.

Drosophila melanogaster stock, $y w^a/w^+Y$ (y , yellow; w^a , white-apricot) was raised at 22°C on standard medium. Imaginal disks (with attached brain) and fat body were dissected from late third instar larvae in *Drosophila* Ringer's. Adult flies with abdomens removed were also used to prepare extracts. Imaginal disks from about 80 larvae, fat body from 15 female or 25 male larvae, or the anterior end of eight adults comprised a single sample. Tissues were transferred to a 1.5 ml Brinkman–Eppendorf microtest tube and homogenised in 0.125 ml of distilled water with a Teflon homogeniser. The homogenates were allowed to stand on ice for 20 min, centrifuged at 12,000g, and the supernatant was used as the extract for enzyme assays. Assay conditions for G6PD, 6PGD, α -GPDH and IDH were as described previously¹⁹. AO assay conditions were those of Dickinson²⁰. Normalised activity measurements were obtained by dividing X-linked gene activity by autosomal gene activity for each extract. The mean ratio is given $\pm$ s.e. with the number of extracts in parentheses. The male/female values are ratios of the means.

amounts of template in males versus females are not adequate to account for dosage compensation in *Drosophila*.

We thank Mr James Kitchens for experimental assistance and Dr G. Maroni for critical advice. This work was supported by the US NIH.

RICHARD L. ROEHRDANZ
JOHN C. LUCCHESI

Curriculum in Genetics and
Department of Zoology,
University of North Carolina,
Chapel Hill, North Carolina 27514

Received 24 April; accepted 18 July 1977.

- ¹ Kazanian, H. H., Jr, Young, W. J. & Childs, B. *Science* **150**, 1601–1602 (1965).
- ² Mukherjee, A. S. & Beermann, W. *Nature* **207**, 785–786 (1965).
- ³ Korge, G. *Proc. natn. Acad. Sci. U.S.A.* **72**, 4550–4554 (1976).
- ⁴ Maroni, G. & Plaut, W. *Genetics* **74**, 331–342 (1973).
- ⁵ Lucchesi, J. C. *Ann. Rev. Genet.* **7**, 225–238 (1973).
- ⁶ Berendes, H. C. *Chromosoma* **20**, 32–43 (1966).
- ⁷ Lakhotia, S. C. & Mukherjee, A. S. *J. Cell Biol.* **47**, 18–33 (1970).
- ⁸ Blumenthal, A. B., Kriegstein, H. J. & Hogness, D. S. *Cold Spring Harb. Symp. quant. Biol.* **38**, 205–233 (1974).
- ⁹ Dolfini, S. A., Courgeon, A. M. & Tiepolo, L. *Experientia* **26**, 1020–1026 (1971).
- ¹⁰ Ananiev, E. V., Palakarova, L. G. & Yurov, Y. B. *Chromosoma* **59**, 259–272 (1977).
- ¹¹ Aronson, J. F., Rudkin, G. T. & Schultz, J. J. *Histochem. Cytochem.* **2**, 258–259 (1954).
- ¹² Rudkin, G. T. in *The Nucleohistones* (eds Bonner, J. & Tso, P.) 184–192 (Holden-Day, San Francisco, 1964).
- ¹³ Hennig, W. & Meer, B. *Nature new Biol.* **233**, 70–72 (1971).
- ¹⁴ Spear, B. B. & Gall, J. G. *Proc. natn. Acad. Sci. U.S.A.* **70**, 1359–1363 (1973).
- ¹⁵ Laird, C. D. & Chooi, W. Y. *Chromosoma* **58**, 193–218 (1976).
- ¹⁶ Lamb, M. M. & Laird, C. D. *Biochem. Genet.* **14**, 359–371 (1976).
- ¹⁷ van de Flierdt, K. *Chromosoma* **50**, 431–434 (1975).
- ¹⁸ Ashburner, M. *Adv. Insect Physiol.* **7**, 1–95 (1970).
- ¹⁹ Lucchesi, J. C. & Rawls, J. M. *Biochem. Genet.* **9**, 41–51 (1973).
- ²⁰ Dickinson, W. J. *Genetics* **66**, 487–496 (1970).

Endocytosis of red blood cells or haemoglobin by activated macrophages inhibits their tumoricidal effect

MICE with chronic *Bacillus Calmette-Guérin* (BCG) or *Toxoplasma gondii* infection have increased resistance to tumour growth, and peritoneal macrophages from these mice can selectively kill neoplastic cells *in vitro* by non-phagocytic means^{1,2}. Although the actual molecular mechanism of killing is unknown, the tumoricidal macrophage (TM) vacuolar system is probably involved. Phase contrast³ and electron microscopy studies⁴ have suggested the translocation of TM lysosomes into tumour cells with subsequent tumour cell death. Trypan blue, an inhibitor of lysosomal enzymes, or hydrocortisone, a membrane stabiliser and inhibitor of membrane fusion, will suppress the *in vitro* tumour cell killing by TM (ref. 3). We report here that endocytosis of red blood cells (RBC), haemoglobin or haemoglobin degradation products by TM inhibits their tumoricidal effect.

In vitro macrophage-mediated tumour cell killing was assayed as described previously³ with some modifications as described in the legend to Fig. 1. Tumour cell killing was assessed by microscopic examination or by measurement of tritiated thymidine (³HTdR) release from pre-labelled 3T12 cells⁵. The results were comparable using TM from either BCG- or *T. gondii*-infected mice.

Results of a typical experiment are shown in Fig. 1. Control TM destroyed very nearly all the 3T12 cells (Fig. 1a). But if the TM had phagocytosed RBC, the killing was markedly suppressed. In Fig. 1b, with an RBC:TM ratio of 4:1, there is complete inhibition of the killing. The inhibition seems to be proportional to the RBC:TM ratio with less complete suppression of killing apparent in Fig. 1c and d with RBC:TM ratios of 2:1 and 1:1, respectively. Experiments using a ³HTdR release assay for tumour killing gave comparable results. For example, the ³HTdR release expressed as a percentage of maximal release (100% release with no RBC added) was $3.2 \pm 2.0\%$, $9.5 \pm 3.4\%$, and $14.4 \pm 2.7\%$ for RBC:TM ratios of 3.00:1, 1.50:1, and 0.75:1 respectively. Figure 2 shows the microscopic features of the inhibitory effects of RBC.

Inhibition was seen when RBC were phagocytosed by the TM. Heat-damaged, glutaraldehyde-treated⁷, or antibody-opsonised⁸ (Cordis Laboratories, rabbit IgG anti-sheep RBC) RBC were all

avidly phagocytosed (>90% of the TM contained ≥ 2 RBC at an RBC:TM ratio of 4:1). If fresh bovine RBC and serum, or fresh mouse RBC and serum syngeneic to the TM (C₃H-He) were present throughout the assay, no TM phagocytosis of RBC occurred. In these situations, there was no inhibition of tumour killing despite close contact between RBC and TM. Of the species examined (human, bovine, rabbit, mouse, chicken, sheep), none was tested that did not suppress killing as long as the RBC were phagocytosed. Syngeneic RBC inhibited if they were phagocytosed. The TM remained viable after the phagocytosis as judged by Trypan blue exclusion, absence of release of the cytoplasmic enzyme lactic dehydrogenase (LDH) (refs 9, 10) and maintenance of their adherence to plastic. TM that had phagocytosed RBC in pre-incubation (with free RBC then washed away, also had diminished tumoricidal effect. *In vivo* loading of TM by intraperitoneal injection of glutaraldehyde-treated RBC 3 d before the assay also resulted in TM with suppressed tumour killing ability.

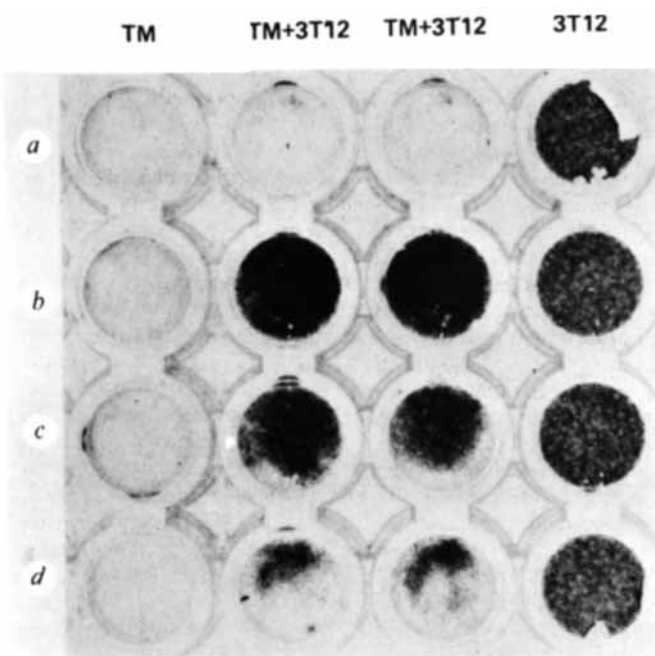


Fig. 1 Photograph of giemsa-stained assay of macrophage-mediated tumor cell killing demonstrating inhibition by red blood cells (RBC). Swiss Webster female mice were infected with either the Paris strain of BCG intraperitoneally (i.p.)³, or the C56 strain of *Toxoplasma gondii* i.p.⁶. At 2–3 weeks after BCG infection or 8–24 weeks after toxoplasma infection, the mice were injected with 2 ml of sterile 10% peptone i.p., and 3 d later the peritoneal cells were collected. Peritoneal cells (4×10^5) in 0.1 ml of Dulbecco's modification of Eagle medium supplemented with streptomycin ($100 \mu\text{g ml}^{-1}$), penicillin (100 U ml^{-1}), HEPES buffer (0.02 M), and glucose (1 mg ml^{-1}) (DMM) were placed into the microtitre wells (Falcon plate 3040) and incubated for 60 min at 37°C in humidified 95% air–5% CO_2 . The nonadherent cells were removed then by washing twice with 0.15 M NaCl (saline). Human RBC were obtained from heparin-anticoagulated blood and washed three times with saline. The RBC were fixed for 30 min at room temperature in 1% glutaraldehyde–saline and washed five times with 15–20 volumes of saline each time. After removal of nonadherent peritoneal cells, 3T12 tumour cells (6×10^3 per well) and appropriate RBC additions were applied in DMM with 10% foetal calf serum. The plates were incubated for 60 h at 37°C in humidified 95% air–5% CO_2 , fixed, and stained with giemsa. Multilayers of 3T12 cells stain dark while TM are not grossly stained. The defects in the tumour cell multilayer apparent in the '3T12' column on rows a and d are artefacts due to detachment and rolling up of the multilayer from the substrate. Row a, (control, no RBC added) reveals the tumoricidal effect of the macrophages seen in the 'TM + 3T12' wells when compared to the control '3T12' well which has no TM. Numbers of RBC in test wells are b, 1×10^6 ; c, 5×10^5 and d, 2.5×10^5 . b–d Demonstrate the RBC inhibition of TM-mediated 3T12 killing with grossly visible multilayers of 3T12 in the 'TM + 3T12' wells. Microscopic examination of a '3T12' well stained 3 h after seeding 6×10^3 3T12 (not shown) reveals 33–37 cells per 300 power field. The 'TM + 3T12' wells in (a) have 0–3 3T12 cells per 300 power field. But, in (d), killing is less complete with many fields having more than three tumour cells and several having a multilayer. Virtually all fields in b and c have multilayers of 3T12 cells.

The act of phagocytosis, *per se*, along with all the accompanying metabolic consequences¹¹, seemed not to be responsible for the RBC-induced inhibition of tumour killing. Latex spheres, either 5.7- μm diameter styrene divinyl benzene (SDB) or 1.01- μm diameter polystyrene (Dow Chemical), were avidly endocytosed by the TM. There was, however, no inhibition of tumoricidal activity until the sphere:TM ratio exceeded 1000:1 for the polystyrene spheres or 100:1 for the SDB spheres. At these ratios, the TM were clearly dead as evidenced by Trypan blue uptake and LDH release. For example, at an SDB:TM ratio of 3:1, there was no inhibition of 3T12 killing and 0% extracellular release of TM LDH, whereas there was complete inhibition of 3T12 killing at an SDB:TM ratio of 100:1 with 61.4% release of the total TM LDH.

Red blood cell ghosts that were phagocytosed after glutaraldehyde treatment or antibody opsonisation did not inhibit killing even at a ghost: TM ratio of 100:1. But, endocytosed¹² ghost-free RBC lysate or chromatographically separated haemoglobin (Hb) was inhibitory at Hb levels $\geq 250 \mu\text{g ml}^{-1}$ (either native or heat denatured). Haem-free globin, prepared by acid-acetone extraction of Hb (ref. 13), was quite unstable in culture media at 37 °C and precipitated as 0.5–1.0- μm diameter spherules. These very hydrophobic globin particles were endocytosed by TM and inhibited tumour killing at levels of 500–1000 $\mu\text{g ml}^{-1}$. Methaemalbumin prepared as described by Tenhunen *et al.*¹⁴ was inhibitory at levels of 500–2,000 $\mu\text{g ml}^{-1}$, whereas albumin treated comparably but lacking haem had no effect on tumour killing. To determine whether the iron of the haem was responsible for the inhibition, ferric chloride (FeCl_3), ferrous sulphate (FeSO_4), and iron dextran (Imferon) were examined. Imferon (200–1,000 μg elemental iron per ml) or FeCl_3 or FeSO_4 (1–40 $\mu\text{g ml}^{-1}$), when left in through the killing assay or used in a pretreatment fashion for 3 h in serum-containing media, completely inhibited the killing by TM. Dextran (average molecular weight 40,000) had no suppressive effect. Non-haemoglobin erythrocyte proteins did not inhibit killing. As much as 1,000 $\mu\text{g ml}^{-1}$ endotoxin-free superoxide dismutase (SOD, Truett Laboratories), catalase (Boehringer), or bovine serum albumin (Pentex)¹⁵ did not suppress macrophage-mediated tumour cell killing. Enhancement of endocytosis of SOD, catalase, or albumin by heat denaturation (120 °C for 20 min followed by sonication if necessary to disperse the denatured proteins) likewise had no effect. Native or heat denatured myoglobin did not inhibit. Table 1 summarises the suppressive effects of various additives.

These results suggest that RBC, Hb, globin, and iron inhibit the tumoricidal effect of activated macrophages. Because damaged

Table 1 Effects of various additions on macrophage-mediated tumour cell killing

Addition	Tumoricidal effect
None	4+
RBC (phagocytosed)*	0
RBC (not phagocytosed)*	4+
RBC ghosts† (phagocytosed)*	4+
RBC lysate‡	0
Haemoglobin‡§	0
Myoglobin‡	4+
Methaemalbumin	0
Albumin‡	4+
Globin‡	0
FeSO_4 or FeCl_3	0
Iron dextran (Imferon)‡	0
Catalase‡	4+
Superoxide dismutase‡	4+

Additions were tested throughout the 60 h *in vitro* assay or in a 2 h pretreatment of TM before tumour cell challenge. All were negative for endotoxin as judged by the limulus amoebocyte lysate test¹⁶. The tumoricidal effect was judged from microscopic assay of microtitre plates stained after 60 h culture³. 4+ Tumoricidal effect signifies 033T12 cell per 300 power field among the TM; zero tumoricidal effect signifies a multilayer of 3T12 cell per 300 power field among the TM.

*RBC TM ratio of 4:1.

†Prepared by hypotonic lysis¹⁷.

‡1000 $\mu\text{g ml}^{-1}$.

§ The haemoglobin was prepared by the method of Haut *et al.*¹⁸, using carboxymethyl cellulose (CM 52, Whatman), eluting the haemoglobin with 0.25 M phosphate buffer, pH 6.8.

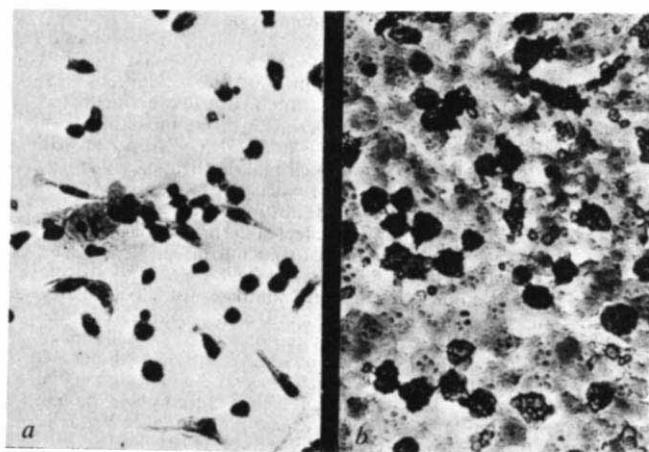
||20 $\mu\text{g ml}^{-1}$.

RBC, Hb, globin, or iron rapidly accumulated in macrophage secondary lysosomes, we interpret our findings as further evidence for involvement of the TM vacuolar system in the non-phagocytic tumour killing process. Vacuolar system and/or plasma membrane damage by iron-induced lipid peroxidation¹⁹, reaction with -SH groups²⁰, or interference with Ca^{2+} transport²¹ may account for the suppressed activated macrophage tumoricidal capability. The lack of inhibition by myoglobin and catalase (which also contain haem moieties with iron) may relate to substrate specificity of haem oxygenase with less or more gradual liberation of 'free' iron from these proteins in the macrophage vacuolar system. Haem oxygenase has little or no degradation effect on myoglobin²². The inhibitory effect of globin is not fully understood, but may relate to its hydrophobic nature. Further elucidation of the mechanisms of the inhibitory effects of RBC, Hb, and Hb degradation products may lead to a more basic knowledge of macrophage-mediated tumour killing. In addition, these observations may aid in understanding the pathogenesis of tumour development in certain clinical situations in man.

There is evidence that iron may contribute to tumour growth in certain experimental and clinical conditions (the occurrence of sarcomas at sites of repeated parenteral iron injections in animals²³ and man²⁴; the increased incidence of hepatomas and other epithelial malignancies in idiopathic haemochromatosis²⁵, acute myeloblastic leukaemia in idiopathic refractory sideroblastic anaemia²⁶, lung carcinoma in mice inhaling iron oxide dust²⁷, and lung carcinoma in haematite miners²⁸). The mechanism by which iron promotes growth of tumours *in vivo* is complex and poorly understood. Some have proposed that iron may act as a primary carcinogen by inducing mutations in somatic cells²⁹, while others have noted a potentiating or cocarcinogenic effect of ferric oxide on chemical carcinogen-induced lung tumours in hamsters³⁰. In addition, some have noted stimulation of tumour cell growth *in vitro* by FeCl_3 (ref 31). Our observed inhibition of the tumoricidal effect of activated macrophages by RBC, haemoglobin, or iron cannot be accounted for by direct effects on tumour cells by the additives, since the inhibition was observed if macrophages had endocytosed the RBC, Hb, or iron before addition of the tumour target cells to the cultures (see Table 1). Therefore, based on the results reported here, we believe that iron could operate through an additional mechanism (inhibition of macrophage tumour killing function) to decrease *in vivo* resistance to tumour development.

Activated macrophages may be the effector component of a

Fig. 2 Photomicrographs from giemsa-stained 'TM+3T12' wells of cytotoxicity assay demonstrating RBC inhibition of macrophage-mediated tumour cell killing. Pictures were made at 60 h from a single experiment as described in Fig. 1 legend. *a*, The TM without added RBC have destroyed the majority of the 3T12 cells. This typical field shows one residual tumour cell among the TM. *b*, The TM with phagocytosed RBC did not kill the 3T12 cells. This typical field shows numerous 3T12 cells with their prominent nucleoli among the RBC-laden TM ($\times 200$).



surveillance system for the detection and destruction of newly emergent clones of neoplastic cells³². Pathologic conditions which produce large amounts of RBC, Hb, or Hb degradation products within the macrophage vacuolar system could suppress tumour cell killing and effectively paralyse this surveillance system.

This work was supported by the Medical Research Service of the Veterans Administration, and by USNIH (grants CA14045 and CA15811). We thank C. C. Moore, M. S. Knowlton, C. Henderson, J. E. Brisbay and R. Christensen for technical assistance.

J. BRICE WEINBERG
JOHN B. HIBBS, JR

Veterans Administration Hospital and
Division of Hematology/Oncology,
Division of Infectious Diseases,
Department of Medicine,
University of Utah Medical Center,
Salt Lake City, Utah 84148

Received 23 May; accepted 5 July 1977.

- ¹ Hibbs, J. B., Jr, Lambert, L. H., Jr & Remington, J. S. *Nature new Biol.* **235**, 48-50 (1972).
- ² Evans, R. & Alexander, P. in *Immunobiology of the Macrophage* (ed. Nelson, D. S.) 536-573 (Academic, London, 1976).
- ³ Hibbs, J. B., Jr *Science* **184**, 468-471 (1974).
- ⁴ Bucana, C. *et al. Cancer Res.* **36**, 4444-4458 (1976).
- ⁵ Norbury, K. C. & Fidler, I. J. *J. immun. Meth.* **7**, 109-122 (1975).
- ⁶ Hibbs, J. B., Jr, Lambert, L. H., Jr & Remington, J. S. *J. infect. Dis.* **124**, 587-592 (1971).
- ⁷ Rubinstein, M. *Proc. Soc. exp. Biol. Med.* **124**, 396-399 (1967).
- ⁸ Bianco, C., Griffin, F. M., Jr & Silverstein, S. C. *J. exp. Med.* **141**, 1278-1290 (1975).
- ⁹ Weissman, G., Dukor, P. & Zurier, R. B. *Nature new Biol.* **231**, 131-135 (1971).
- ¹⁰ Allain, C. C., Henson, C. P., Nadel, M. K. & Knobelsdorff, A. J. *Clin. Chem.* **19**, 223-227 (1973).
- ¹¹ Rossi, F., Romeo, D. & Patriarca, P. *J. reticuloendothel. Soc.* **12**, 127-149 (1972).
- ¹² Ehrenreich, B. A. & Cohn, Z. A. *J. Cell Biol.* **38**, 244-248 (1968).
- ¹³ Rossi-Fanelli, A., Antonini, E. & Caputo, A. *Biochim. biophys. Acta* **30**, 608-615 (1958).
- ¹⁴ Tenhunen, R., Marver, H. S. & Schmid, R. *Proc. natn. Acad. Sci.* **61**, 748-755 (1968).
- ¹⁵ Ehrenreich, B. A. & Cohn, Z. A. *J. exp. Med.* **126**, 941-958 (1967).
- ¹⁶ Elin, R. J., Sandberg, A. L. & Rosenstreich, D. L. *J. Immun.* **117**, 1238-1242 (1976).
- ¹⁷ Dodge, J. T., Mitchell, C. & Hanahan, D. J. *Archs Biochem. Biophys.* **100**, 119-130 (1963).
- ¹⁸ Haut, A., Cartwright, G. E. & Wintrobe, M. M. *J. Lab. clin. Med.* **63**, 278-289 (1964).
- ¹⁹ Arstila, A. U., Smith, M. A. & Trump, B. F. *Science* **175**, 530-533 (1971).
- ²⁰ Jocelyn, P. C. (ed.) *Biochemistry of the SH Group* 94-115 (Academic, New York, 1972).
- ²¹ Romslo, I. & Flatmark, T. *Biochim. biophys. Acta* **325**, 38-46 (1973).
- ²² Tenhunen, R., Marver, H. S. & Schmid, R. *J. Biol. Chem.* **244**, 6388-6394 (1969).
- ²³ Haddow, A. & Horning, E. S. *J. natn. Cancer Inst.* **24**, 109-127 (1960).
- ²⁴ MacKinnon, A. E. & Bunciewicz, J. *Br. med. J.* **2**, 277-279 (1973).
- ²⁵ Bomford, A., Walker, R. J. & Williams, R. in *Iron Metabolism and Its Disorders* (ed. Kief, H.), 324-331 (Elsevier, New York, 1975).
- ²⁶ Kushner, J. P., Lee, G. R., Winbroke, M. M. & Cartwright, G. E. *Medicine* **50**, 139-159 (1971).
- ²⁷ Campbell, J. A. *Br. med. J.* **2**, 275-280 (1940).
- ²⁸ Boyd, J. T., Doll, R., Faulds, J. C. & Leiper, J. *Br. J. ind. Med.* **27**, 97-105 (1970).
- ²⁹ Richmond, H. G. *Br. J. Cancer* **15**, 594-606 (1961).
- ³⁰ Montesano, R., Suffiotti, U. & Shubick, P. in *Inhalation Carcinogenesis* (eds Hanna, M. G., Nettesheim, P. & Gilbert, J.) 353-375 (AEC Symposium Series 18, Oak Ridge, Tennessee, 1970).
- ³¹ Brun, A. & Brunk, U. *Cytobiologie* **12**, 417-428 (1976).
- ³² Hibbs, J. B., Jr, Lambert, L. H., Jr & Remington, J. S. *Science* **177**, 998-1000 (1972).

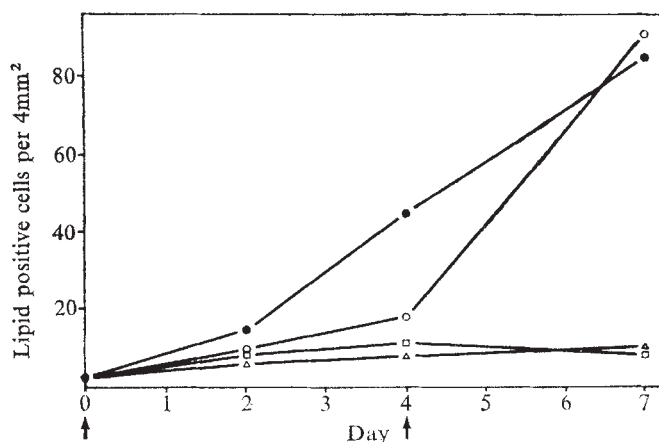


Fig. 1 Effect of TPA on adipose conversion of clone A31T fibroblasts. Cells were seeded into gridded 60-mm plastic Petri dishes at a density of 4×10^4 cells per cm^2 . After 3-4 d, when the monolayers were confluent, and again on day 4, the cultures were refurnished ($\uparrow$) with fresh medium containing insulin $0.1 \mu\text{g ml}^{-1}$ and TPA. The number of lipid-positive cells per 4 mm^2 was scored by microscopic observation without staining every 2-3 d and confirmed after staining with oil red O at the termination of the experiment. ●, Control; □, TPA $1.6 \times 10^{-7} \text{ M}$; ○, TPA $1.6 \times 10^{-9} \text{ M}$.

held as confluent monolayers, the cells begin to accumulate lipids. They soon become mature adipocytes in which the cytoplasm is filled with lipid droplets and lose the ability to divide; after a few weeks, more than half the cells in the monolayer may be mature adipocytes. Both immature and mature adipose cells are easily identified either without staining or after staining with the lipid-specific stain oil red O. Stock cultures of clone A31T were grown in Eagle's basal medium supplemented with 10% foetal calf serum and were subcultured at 1:10 ratios just as the monolayers reached confluence, so that adipose conversion was kept at a minimum.

TPA and 4 α -phorbol-12,13-didecanoate (4 α -PDD) were purchased from Consolidated Midland; phorbol-12,13-didecanoate (PDD), phorbol-12,13-dibenzoate (PDB) and phorbol-12,13-diacetate (PDA) were the gift of Dr R. K. Boutwell, University of Wisconsin. Stock solutions ($1.6 \times 10^{-4} \text{ M}$ in acetone) of the phorbol diesters were stored at -20°C and diluted in medium as required.

The effect of TPA on adipose conversion in clone A31T fibroblasts is shown in Fig. 1. In control cultures, the number of lipid-positive cells increased at the rate of approximately 15 cells per 4 mm^2 per d whereas in cultures treated with $1.6 \times 10^{-7} \text{ M}$ or $1.6 \times 10^{-9} \text{ M}$ TPA, there was no increase in the number of lipid-positive cells during with 7-d period of observation. With a 10-fold lower concentration of TPA, adipose conversion was inhibited for the first 4 d, but by 7 d the number of lipid-positive cells was similar to the number in control cultures.

The inhibitory effect of TPA was reversible after 7 d by refeeding the cultures with control medium. The number of lipid-positive cells increased thereafter at approximately the same rate as in control cultures never exposed to TPA.

The inhibitory effects of TPA on adipose conversion in clone A31T were similar whether or not insulin was in the medium, although insulin did enhance conversion^{11,12}. In one experiment, after 10 d the number of lipid-positive cells in control cultures with insulin $0.1 \mu\text{g ml}^{-1}$ was 82 per 4 mm^2 and without insulin, 58 per 4 mm^2 ; the numbers in TPA ($1.6 \times 10^{-7} \text{ M}$)-treated cultures were 2.1 and 2.8, respectively.

As has been reported with other 3T3 cell lines^{13,14}, TPA stimulated multiplication of clone A31T cells and increased the saturation density of the monolayers. Thus,

Inhibition of adipose conversion of 3T3 fibroblasts by tumour promoters

TUMOUR-PROMOTING compounds are not themselves carcinogenic, but can induce skin tumours in mice pre-treated with a subcarcinogenic dose of a chemical carcinogen¹⁻³. They have been shown to produce many different biochemical and biological effects on cells^{3,4}, but the mechanism of promotion is not known. Two recent reports describe the effects of the phorbol diester series of tumour promoters on cell differentiation *in vitro*. Cohen *et al.*⁵ observed that 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA); phorbol myristate acetate, a potent promoter, inhibits myogenesis in chick embryo muscle cultures. We have reported^{6,7} that TPA and other tumour-promoting phorbol diesters inhibit spontaneous and induced differentiation of Friend erythroleukaemia cells in culture. We now describe another cell system, mouse preadipose cells, in which tumour promoters inhibit terminal differentiation, and confirm in these cells our original observation⁶ that there is a good correlation between the ability of a particular phorbol diester to promote tumours in mouse skin and its ability to inhibit terminal differentiation of cells in culture.

A clone of BALB/c 3T3 fibroblasts⁸ was obtained from Dr R. Tennant, Oak Ridge National Laboratory. In our laboratory, cells of this clone (designated clone A31T) behave as preadipose cells and differentiate to mature adipocytes in a manner similar to that already seen in some clones of Swiss 3T3 fibroblasts⁹⁻¹². When cultures are

at the termination of the experiment shown in Fig. 1, cultures treated with 1.6×10^{-7} M TPA had 3.2×10^6 cells per plate, 0.15% of which were lipid positive, whereas control plates had 1.6×10^6 cells with 2.7% lipid positive.

The effects on adipose conversion of a series of phorbol diesters with a range of tumour-promoting activity in mouse skin^{2,15,16} are shown in Fig. 2. At the same concentration (1.6×10^{-7} M), the three promoters (TPA, PDD and PDB) completely inhibited adipose conversion whereas the three non-promoters (PDA, 4 α -PDD and phorbol) had no effect on conversion. The three active promoters also enhanced the saturation density of the cell monolayers approximately twofold; the three non-promoters had no effect on cell number.

The inhibitory effect of the promoters on adipose conversion was not permanent, even when the cultures were refurbished with fresh solutions of the phorbol diesters every 3-4 d. In five separate experiments, the inhibition of conversion by TPA (1.6×10^{-7} M) persisted for 14-18 d (Table 1), after which time the number of lipid-positive cells slowly increased. PDD and PDB, however, inhibited adipose conversion for only 7 d; after that time there was a rapid and sudden increase in adipose conversion. In the experiment shown in Table 1, for example, the number of lipid-positive cells per 4 mm² in PDD-treated cultures increased from 0 on day 7 to 184 on day 10. In PDB-treated cultures, there was an increase from 1.2 to 167 positive cells. These increases in adipose conversion first became evident on days 8 to 9 when there seemed to be an almost synchronous increase in the number of immature adipocytes; a few days later, these cells were fully mature adipocytes filled with lipid. Following this spontaneous release from the inhibitory effects of PDD and PDB, the percentage of lipid-positive cells gradually approached the percentage in control cultures (Table 1). Thus, on day 14 the absolute number of lipid-positive cells in PDD- and PDB-treated cultures was greater than in control cultures, but since the treated cultures had almost twice as many cells, the percentage of lipid-positive cells in the three groups was similar.

The finding that tumour-promoting phorbol diesters inhibit terminal differentiation in cultures of three different cell types, erythroleukaemia cells^{6,7}, muscle cells⁸ and preadipose cells, suggests that inhibition of terminal differentiation of cells in culture may be a general property of tumour promoters. They inhibit differentiation reversibly at concentrations that are not cytotoxic and that either stimulate or have no effect on cell division.

The phorbol diesters that inhibit adipose conversion of

Fig. 2 Effect of phorbol diesters on adipose conversion of clone A31T fibroblasts (Fig. 1 for procedure). The phorbol diesters were all tested at the same molar concentration, 1.6×10^{-7} M. ●, Control; △, TPA; ▲, PDD; X, 4 α -PDD; □, PDB; ○, PDA; ■, phorbol.

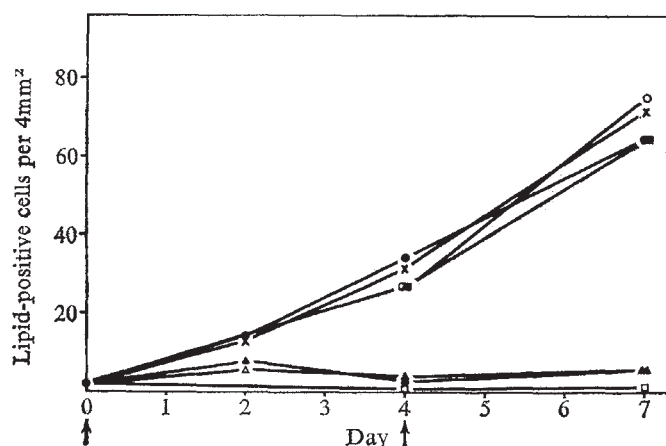


Table 1 Long-term effects of phorbol diesters on adipose conversion

Number of lipid-positive cells per 4 mm ²				
Day	Control	TPA	PDD	PDB
3	3.7±3.0*	0	0	0
7	106±15	0.5±0.9	0	1.2±2.4
10	169±15	12±2.3	184±22	168±22
14	223±30	3.4±1.2	368±65	>500
17	240±52	6.1±4.3	—	—

% lipid-positive cells				
Day	Control	TPA	PDD	PDB
3	0.1	0	0	0
7	2.7	<0.1	0	<0.1
10	4.8	0.1	3.0	2.0
14	6.3	<0.1	6.0	6.8

Cultures of clone A31T fibroblasts were refurbished on days 0, 4, 7, 11 and 14 with medium containing the phorbol diesters at a final concentration of 1.6×10^{-7} M. On day 0, there were 2.1×10^6 cells per plate. By day 3, the cell monolayers had attained densities that remained essentially unchanged through day 14. The mean number of cells ($\times 10^{-6}$) per plate $\pm$ s.d. from days 3-14 was: control, 2.0 ± 0.3 ; TPA, 3.9 ± 0.1 ; PDD, 3.3 ± 0.4 ; PDB, 4.0 ± 0.5 .

*Mean $\pm$ s.d. of five squares.

clone A31T cells stimulate cell division the first time they are added to confluent cultures. The cells seem to retain their density-dependent growth controls during repeated refurbishing of the cultures with fresh solutions of phorbol diesters; the saturation densities remain essentially constant without cells being shed into the medium. This suggests that inhibition of differentiation in these cells may not be dependent on continuous cell cycling.

The reasons for the spontaneous release of clone A31T cells from the inhibitory effects of the promoters are not known. In contrast to these cells, Friend erythroleukaemia cells do not seem to become refractory to the inhibitory effects of the promoters on spontaneous differentiation; the extent of inhibition has remained the same in erythroleukaemia cells maintained for six months in the continuous presence of TPA⁷.

Tumour promoters induce many diverse effects on cells in culture⁴. It must be determined whether the inhibitory effects of promoters on differentiation are related to any of the other effects or to the mechanism of promotion itself. The ability of promoters to shift the cell population from a commitment to differentiate and become end cells to a commitment to maintain or increase the size of the stem cell pool, does suggest at least one possibility for the mechanism of promotion. The inhibition of terminal differentiation by tumour promoters may give cells in which the potential for malignancy has been initiated by a carcinogen, but is not yet expressed, additional time to convert to tumour cells before becoming end cells. Several investigators^{17,18} have proposed that tumour promoters act *in vivo* by interfering with normal cell differentiation. Our findings support those suggestions.

This work was supported by the NIH. G.R. is a Scholar of the Leukemia Society of America. T.G.O'B is a USPHS postdoctoral fellow.

LEILA DIAMOND
THOMAS G. O'BRIEN
GIOVANNI ROVERA

Wistar Institute of Anatomy and Biology,
36th Street at Spruce,
Philadelphia, Pennsylvania 19104

Received 13 May; accepted 18 July 1977.

¹ Van Duuren, B. L. *Progr. exp. Tumor Res.* 11, 31-68 (1969).

² Hecker, E. *Methods Cancer Res.* 6, 439-484 (1971).

³ Boutwell, R. K. *C.R.C. Crit. Rev. Toxic.* 2, 419-443 (1974).

- 4 Weinstein, I. B., Wigler, N. & Pietropaolo, C. in *Cold Spring Harbor Symposium on the Origins of Human Cancer* (Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, in the press).
- 5 Cohen, R., Pacifici, M., Rubinstein, N., Biehl, J. & Holtzer, H. *Nature* **266**, 538-540 (1977).
- 6 Rovera, G., O'Brien, T. & Diamond, L. *Proc. natn. Acad. Sci. U.S.A.* **74**, 2894-2898 (1977).
- 7 Diamond, L., O'Brien, T. & Rovera, G. in *Symposium on Mechanisms of Tumor Promotion and Cocarcinogenesis* (Raven, New York, in the press).
- 8 Quarles, J. M. & Tennant, R. W. *Cancer Res.* **35**, 2637-2645 (1975).
- 9 Green, H. & Kehinde, O. *Cell* **1**, 113-116 (1974).
- 10 Green, H. & Meuth, M. *Cell* **3**, 123-133 (1974).
- 11 Green, H. & Kehinde, O. *Cell* **5**, 19-27 (1975).
- 12 Russell, T. R. & Ho, R.-J. *Proc. natn. Acad. Sci. U.S.A.* **73**, 4516-4520 (1976).
- 13 Sivak, A. & Van Duuren, B. L. *Cancer Res.* **30**, 1203-1205 (1970).
- 14 Diamond, L., O'Brien, S., Donaldson, C. & Shimizu, Y. *Int. J. Cancer* **13**, 721-730 (1974).
- 15 Thielmann, H. W. & Hecker, E. in *Fortschritte der Krebsforschung* (eds Schmidt, C. G. & Wetter, O.) 171-179 (Schattauer, Stuttgart and New York, 1969).
- 16 Baird, W. M. & Boutwell, R. K. *Cancer Res.* **31**, 1074-1079 (1971).
- 17 Raick, A. N. *Cancer Res.* **34**, 2915-2925 (1974).
- 18 Marks, F. *Cancer Res.* **36**, 2636-2643 (1976).

Effect of microtubular antagonists on lymphocyte mitogenesis

SEVERAL different classes of mitogens have been discovered¹. Stimulation of lymphocytes by all classes of mitogens results in cellular division, but it is not known whether all mitogens act on the cell membrane in identical fashion or result in triggering of the same cytoplasmic messenger. We have investigated the inhibitory effect of microtubule disrupting drugs on lymphocytes transformed by two methods. We report here that colchicine and vinblastine inhibit lectin-mediated mitogenesis at low concentrations, while cells stimulated by cell surface oxidation are inhibited only by much higher concentrations of these drugs. It seems that intact microtubules are necessary for lectin-mediated but not for cell surface oxidation-mediated mitogenesis.

Yahara and Edelman have demonstrated that microtubules and microfilaments exist in association with the lymphocyte cell surface². Edelman *et al.* have hypothesised that a colchicine-binding protein system may be a crucial link in the transmission of information from the cell membrane to the nucleus³. In support of this is the finding that drugs which disrupt microtubules block lectin-induced stimulation of lymphocytes at low drug concentrations^{4,5}.

We stimulated lymphocytes with two different classes of mitogens, (1) the plant lectins concanavalin A (con A) and phytohaemagglutinin (PHA) and (2) enzymatic oxidation of the cell surface by means of sequential additions of neuraminidase and galactose oxidase (NG) (ref. 6). Both classes of mitogens are known to selectively stimulate T cells⁷.

The effect of vinblastine on lymphocyte transformation measured by ³H-thymidine uptake is shown in Fig. 1. There is a significant difference between the amount of vinblastine needed to inhibit lectin-mediated stimulation and that amount needed to inhibit NG-treated lymphocytes ($P < 0.02$). Vinblastine had almost no inhibitory effect on NG-treated cells until a concentration of 10^{-5} M was reached. Lectin-treated cells were inhibited by drug concentrations 100 to 1,000-fold less.

The curve describing thymidine uptake as a function of antagonist concentration for NG-treated cells is suggestive of simple saturation kinetics, while the curves for lectin-stimulated cells show evidence of a more complex cooperative interaction. Colchicine gave results similar to those found with vinblastine (Fig. 2). Cytosine arabinoside, a drug known to directly interfere with DNA synthesis¹⁰, inhibits both groups to the same extent while showing similar inhibition kinetics (Fig. 3). This suggests that, not surprisingly, lectin- and NG-mediated stimulation share a common pathway at the level of DNA synthesis.

Table 1 illustrates the $-\log_{10} K_{1/2}$ of colchicine, vinblastine, and cytosine arabinoside for lymphocyte blastogenesis induced by lectins and cell surface oxidation. The significant difference between lectin- and NG-treated cells is evident for both vinblastine and colchicine although vinblastine proved to be a slightly more potent antagonist. Edelman *et al.* have hypothesised that a colchicine-binding

protein system has a role in the mediation of interactions between receptors on cellular membranes and the cytoplasm as well as in mitogenesis³. Microtubular inhibitors also inhibit mitogenesis in neuroblastoma and mouse 3T3 cells as well as in lymphocytes¹¹. Our results support reports^{4,5} that colchicine and vinblastine are effective inhibitors of lymphocyte mitogenesis and do not support the isolated report that vinblastine is not inhibitory¹².

Resch *et al.* have shown that three cellular events which occur in transformed lymphocytes before the initiation of DNA synthesis were not inhibited by colchicine or vinblastine¹³. They concluded that microtubules are not involved in the initiation of lymphocyte activation because the disruption of these cytoplasmic structures should also affect cellular events which occur before DNA synthesis. This conclusion is justified only if those early cellular events

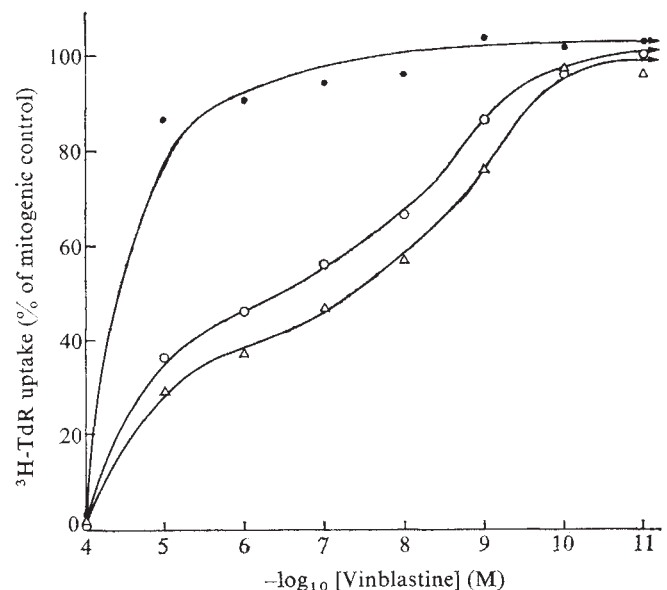


Fig 1. Effect of vinblastine on lymphocyte transformation. Blood was obtained from human donors free of any acute or chronic illnesses and on no medications. Lymphocytes were isolated using the method of Boyum⁸. The cell population was made up of an average of 85% lymphocytes, 12% monocytes and 3% neutrophils. Cell viability following isolation and after treatment with NG or lectin was always over 95% as measured by Trypan blue exclusion and fluorescein diacetate uptake⁹. Cells were cultured in McCoy's 5A Medium (Microbiological Associates) supplemented with 10% autologous serum at a cell density of 1×10^6 cells per ml, in Nunc serum tubes (Vanguard International). The cultures were put in a humidified atmosphere of 5% CO_2 , 95% air at 37 °C. At 48 h, 1 μCi ³H-thymidine (TdR), specific activity 6.7 mCi mmol⁻¹ (New England Nuclear) was added to each culture. At 72 h, the trichloroacetic acid-insoluble product was separated onto 0.45- μm Millipore filters. The dried filters were put into a scintillation cocktail of 0.5 g PPO and 5 g POPOP per l toluene and counted overnight in a Packard Tri Carb Scintillation Counter. Cell surface oxidised lymphocytes were treated with 50 units ml⁻¹ neuraminidase for 30 min at 37 °C at a cell density of 20×10^6 cells per ml. Neuraminidase (EC 3.2.1.18 from *Vibrio cholerae* strain Z-4) was obtained from GIBCO as a solution containing 500 units ml⁻¹ where 1 unit releases 1 μg of N-acetyl neuraminic acid from a glycoprotein substrate in 15 min at pH 5.5 at 37 °C. This was followed by treatment with 0.25 units ml⁻¹ galactose oxidase (Worthington) for 30 min at 23 °C at a cell density of 20×10^6 per ml. A unit is the quantity of enzyme that yields an absorbance of 1.0 at 420 nm by the peroxidase-chromagen assay. PHAMR 69 (Wellcome), con A (Calbiochem); colchicine (Sigma), and vinblastine (Lilly), were added to the cells just before they were put into culture. Each point represents duplicate cultures of 10 different donors. Data is expressed as the mean % of mitogenic control. Absolute c.p.m. for mitogenic controls were 68,500-122,300 for con A, 72,400-131,200 for PHA, and 58,400-118,600 for NG. Mean mitogenic controls were 102,500 for con A, 108,600 for PHA, and 87,400 for NG. ●, NG; ○, PHA; △, con A.

Table 1 Values for $-\log_{10}K_1^{50}$; K_1^{50} is the drug concentration inhibiting mitogenesis by 50%

Mitogen	Vinblastine		N	Colchicine		N	Ara-C		N
Con A	7.45 ± 0.63*		10	6.95 ± 0.66		10	6.60 ± 0.57		5
PHA	6.55 ± 0.42		10	6.50 ± 0.56		10	6.70 ± 0.38		5
NG	4.45 ± 0.46		10	4.45 ± 0.48		10	6.80 ± 0.58		5
	<i>t</i> †	<i>P</i>	<i>t</i>	<i>P</i>	<i>t</i>	<i>P</i>			
Con A versus NG	3.02	<0.02	2.95	<0.02	0.221	>0.2			
PHA versus NG	2.64	<0.05	2.57	<0.05	0.115	>0.2			
Con A versus PHA	0.73	>0.2	0.52	>0.2	0.147	>0.2			

N, number of experiments performed.

*Values are means ± s.e.m.

† *t* values determined by Student's *t*-test.

are directly linked and necessary for lymphocyte activation to take place. No evidence exists to suggest that blocking membrane turnover, early RNA synthesis, or production of lymphotoxin leads to inhibition of lymphocyte activation. Furthermore, it has been shown that colchicine inhibits phosphatidylinositol turnover, another early cellular event, in con A-stimulated lymphocytes¹⁴.

It has been suggested that inhibition of mitogenesis by colchicine is due to blockage of thymidine transport. Wilson¹⁵ found the inhibition constant for blockage of thymidine transport by colchicine to be 6.1×10^{-5} M. Interestingly, in our experiments NG-mediated mitogenesis was not 50% inhibited by colchicine until levels of 5×10^{-5} M were reached. Thus for NG-mediated mitogenesis our results are consistent with, but do not prove, the hypothesis that the site of action of colchicine may be at the level of thymidine transport. Furthermore, saturation kinetics, consistent with competitive inhibition of transport were observed. In contrast, con A-mediated blastogenesis was 50% inhibited by 1×10^{-7} M colchicine and 4×10^{-8} M vinblastine, a concentration far too low to act at the level of thymidine transport. This is close to the purified tubulin affinity constant for adult human brain of 3.4×10^{-7} M (ref. 15).

It has been assumed until now that lectins and cell sur-

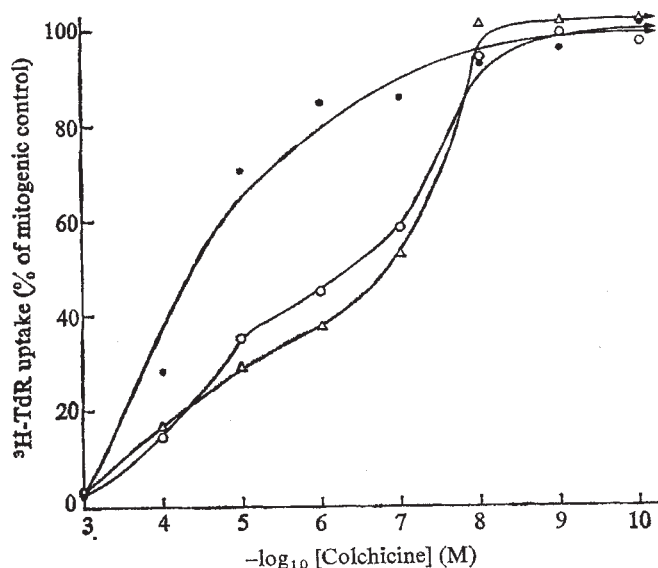


Fig. 2 Effect of colchicine on lymphocyte transformation. Experimental conditions were the same as for Fig. 1. Each point represents duplicate cultures of 10 different donors. Data expressed as in Fig. 1. ●, NG; ○, PHA; △, con A.

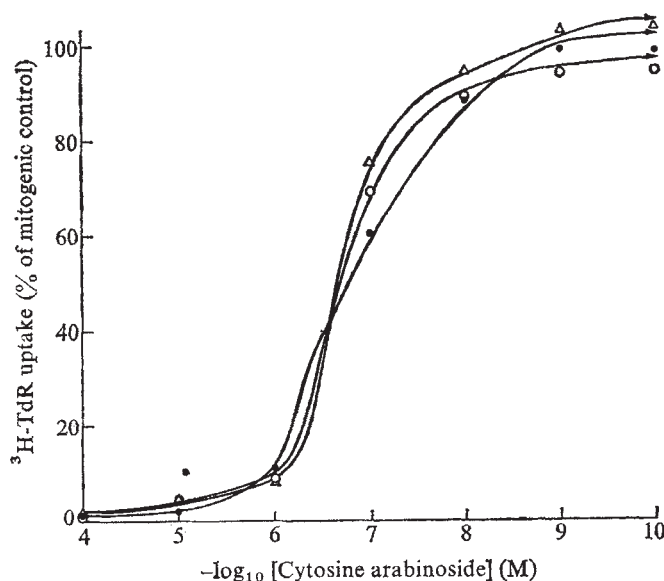


Fig. 3 Effect of cytosine arabinoside on lymphocyte transformation. Experimental conditions as for Fig. 1. Each point represents duplicate cultures of five different donors. Data expressed as in Fig. 1. ●, NG; ○, PHA; △, con A.

face oxidation result in a perturbation of the cellular membrane (perhaps cross-linking^{16,17}) that results in secondary triggering of the same cytoplasmic messenger. It may be, however, that a biological process as complex as mitogenesis is under multifactorial control. There is evidence that the initial membrane event for the two groups is different. Free aldehyde groups generated by cell surface oxidation do not seem to be generated by lectins since mitogenesis due to the former but not the latter stimulus is inhibited by reducing agents⁸. Our results show that although microtubules are necessary for lectin-mediated lymphocyte transformation, they are not a crucial intermediary in transfer of information from cell surface to the genome for cell surface oxidised cells.

STEVEN A. RASMUSSEN
ROBERT P. DAVIS

Division of Biology and Medicine,
Brown University and
Division of Renal and Metabolic Diseases,
The Miriam Hospital,
Providence, Rhode Island 02906

Received 4 April; accepted 5 July 1977.

- O'Brien, R. L. & Parker, J. W. *Cell* 7, 13-20 (1976).
- Yahara, I. & Edelman, G. M. *Expl Cell. Res.* 91, 125-142 (1975).
- Edelman, G. M., Yahara, I. & Wang, J. L. *Proc. natn. Acad. Sci. U.S.A.* 70, 1442-1446 (1973).

- ⁴ Medrano, E., Piras, R. & Mordoh, J. *Expl Cell Res.* **86**, 295–300 (1974).
⁵ Wang, J. L., Gunther, G. R. & Edelman, G. M. *J. Cell Biol.* **66**, 128–144 (1975).
⁶ Novogrodsky, A. & Katchalski, E. *Proc. natn. Acad. Sci. U.S.A.* **70**, 1824–1827 (1973).
⁷ Novogrodsky, A. *Eur. J. Immun.* **48**, 646–648 (1974).
⁸ Boyum, A. *Scand. J. clin. Lab. Invest.* **21**, Suppl. 97, 77–88 (1968).
⁹ Rotman, B. & Papermaster, B. W. *Proc. natn. Acad. Sci. U.S.A.* **55**, 134–141 (1965).
¹⁰ Chu, M. Y. *Biochem. Pharmacol.* **20**, 2057–2063 (1971).
¹¹ Baker, M. E. *Nature* **262**, 785–786 (1976).
¹² Betel, I. & Marijnsse, J. *Nature* **261**, 318–319 (1976).
¹³ Resch, K., Bouillon, D., Gerns, D. & Averdunk, R. *Nature* **265**, 349–351 (1977).
¹⁴ Schellenberg, R. R. & Gillespie, E. *Nature* **265**, 741–742 (1977).
¹⁵ Wilson, L. *Ann. N. Y. Acad. Sci.* **253**, 213–230 (1975).
¹⁶ Anderson, J., Edelman, G. M., Moller, G. & Sjoberg, O. *Eur. J. Immun.* **2**, 233–235 (1972).
¹⁷ Greaves, M. & Janossy, G. *Transplant. Rev.* **11**, 87–130 (1972).

Immunoglobulin binding to herpes virus-induced Fc receptors inhibits virus growth

WATKINS¹ reported that sheep red blood cells coated with anti-sheep-red blood cell antibody adsorbed to monolayers of herpes simplex virus (HSV) infected cells. Yasuda and Milgrom² suggested that the haemadsorption was mediated through an Fc-binding site which developed on the surface of the HSV infected cell, and later work from Watkin's laboratory established this interpretation³. Although the receptor for the Fc portion of IgG on the surface of HSV infected cells has been known for some time, its biological role remains unclear^{4,5}. We show here that when the HSV-induced Fc receptor is engaged with IgG or Fc fragments, viral replication is significantly inhibited.

Pools of HSV-2 (strain MS) were grown and titrated in Vero cell monolayers⁶. The Fc receptor studies were done with Vero monolayers and with a human retinoblastoma cell line (Y-79) which grows in suspension⁷. The cells were grown in RPMI 1640 medium (NIH) with 5% foetal calf serum (GIBCO). Fc receptors were demonstrated with sheep red blood cells coated with rabbit 7S antibody to sheep red cell stroma (7SEA). Sheep red cells coated with 19S antibodies were used as control reagents. Haemadsorption assays were done with minor modifications of previously described methods^{8,9}.

Both Vero and Y-79 cultures infected with HSV-2 contained 20–40% of the cells with Fc receptor 20 h after infection. No haemadsorption was seen in infected cultures with 19S-EA reagent, and uninfected cells were negative with both the 7S-EA and 19S-EA. In the Y-79 cultures infected at a multiplicity of 100, haemadsorption was first detected after 6 h, and by 8 h after infection the percentage of rosetting cells was equal to that detected 20 h after infection. Haemadsorption was inhibited when the infected cells were pre-incubated in balanced salt solution (BSS) containing 1 mg ml⁻¹ of IgG of different species (goat, rabbit, mouse). Haemadsorption inhibition was also achieved with Fc fragments but not with (Fab')₂ fragments of rabbit IgG. These results suggest that IgG and Fc fragments occupy the Fc binding sites at the surface of HSV infected cells. Furthermore radiolabelled IgG is known to bind to HSV infected cells³.

We next tested the effects of IgG on virus growth in these cells. The normal concentration of IgG in human serum is 12 mg ml⁻¹, so to avoid unphysiological levels we used concentrations of 1–10 mg ml⁻¹. Serum-free RPMI 1640 medium was prepared with DEAE-purified rabbit IgG in concentrations of 1, 5 and 10 mg ml⁻¹. The IgG used in all experiments was free of HSV neutralising activity in a standard viral plaque neutralisation test. Bovine serum albumin (Sigma) in 1, 5 and 10 mg ml⁻¹ concentrations was used as control medium. Cells were infected at a multiplicity of 100 for 1 h at 35 °C, washed twice in RPMI 1640, and transferred to IgG- or albumin-containing medium. They were incubated for 24–48 h at 35 °C and the total

virus in the cultures after three cycles of freezing and thawing was assayed on Vero monolayers.

As shown in Table 1, there was a 99% reduction in virus production in the cultures treated with 10 mg ml⁻¹ IgG. There was no inhibition of virus growth in 1 mg ml⁻¹ IgG. These results have been consistently reproducible both in Vero and Y-79 cells.

Although the concentrations of IgG we used were in the physiological range, we still thought it would be of importance to determine whether they were cytotoxic in our cell systems. We tested viability of non-infected Vero and Y-79 cells maintained in our 10 mg ml⁻¹ IgG medium for 24–48 h by Trypan blue exclusion and found no cytotoxicity.

We have also tested the effects of purified Fc fragments of rabbit IgG on virus growth. Rabbit IgG was digested with papain and the Fc fragments were purified by the method of Prahl¹⁰. When infected cultures were maintained in medium with 5 mg ml⁻¹ Fc fragments, virus production was 99% inhibited (Table 1). Similar inhibition was seen with Fc fragments at 2.5 mg ml⁻¹ and no effect was demonstrable with 1 mg ml⁻¹.

The infected monolayers of Vero cells maintained on IgG showed a marked decrease in viral cytopathic effect when examined by light microscopy. Electron microscopic examination of Vero and Y-79 cultures 24 and 48 h after infection in 10 mg ml⁻¹ IgG medium showed a marked decrease in viral capsids in the nuclei when compared to control cultures (Fig. 1). Most of the capsids in the IgG treated cells were empty, and accumulations of empty capsids in the cytoplasm were prominent. The same morphological findings were observed when the infected cultures were incubated in medium containing Fc fragments.

In all of the infected cultures examined by electron microscopy, the morphological effects on virus by IgG and Fc fragments were seen in more than 90% of the cells. Although only 20–40% of the cells in infected cultures produced rosettes with the 7S-EA, it is known that haemadsorption with 7S-EA is not an efficient method for demonstrating Fc receptors in other systems¹¹, and our electron microscopical findings suggest that the immunoglobulin exerts an inhibitory effect on most if not all infected cells in the cultures.

We also tested the effect of incubation in IgG containing media on infectious centre production. Y-79 cells were infected with a multiplicity of 100, washed three times in BSS and 10⁴, 10³ and 10² cells plated on Vero cell monolayers. After plating, the monolayers were fed with IgG-containing medium supplemented with 2% of HSV anti-serum in order to obtain plaques. This experiment showed

Table 1 Effect of rabbit IgG and Fc fragments on HSV growth in retinoblastoma and Vero cells

	Total virus produced (PFU ml ⁻¹)
Retinoblastoma	
IgG (10 mg ml ⁻¹)	2.3 × 10 ⁴
Fc Fragment (5 mg ml ⁻¹)	1.2 × 10 ⁴
Control	1.3 × 10 ⁶
Vero	
IgG (10 mg ml ⁻¹)	1.2 × 10 ²
Fc (5 mg ml ⁻¹)	1 × 10 ³
Control	2.5 × 10 ⁴

Cultures were infected at a multiplicity of 100 and viral growth was measured 24 h (Vero) and 48 h (retinoblastoma) after infection. Control cultures contained equivalent protein concentration of albumin. 10⁴ cells were infected with 10⁶ plaque forming units (PFU) and incubated in IgG or Fc fragment-containing media. At the end of the experiment the number of cells was not changed, indicating no loss of cells or growth during the experiment. The cell viability was 90% by Trypan blue exclusion in both controls and IgG treated cultures.

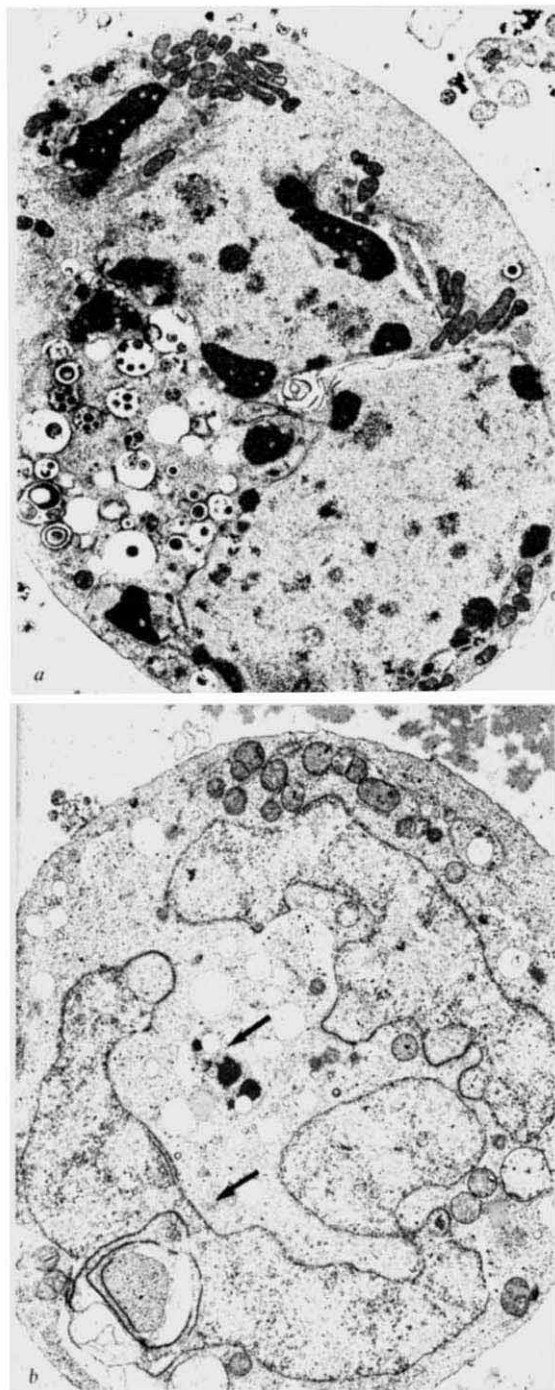


Fig. 1 Electronmicrographs of Vero cells infected with HSV-2. *a*, Cells incubated for 24 h in media containing albumin. Production of complete virions is seen. *b*, Cells incubated with 10 mg ml^{-1} of rabbit IgG. Empty viral capsids are present in the nucleus and cytoplasm. ($\times 17,000$).

no infectious centres in IgG-containing medium with 10^4 , 10^3 or 10^2 cells while controls in medium free of IgG showed that 12% of the infected cells plated as infectious centres. The low percentage of infectious centres in the controls may be related to the use of a fluid rather than semi-solid overlay.

Our results establish that IgG and its Fc fragment inhibit the growth of HSV and strongly suggest that the effect is due to engagement of the virus-induced Fc receptor. Although the mechanism is not yet known, it is possible that this inhibition obtained with physiological concentrations of IgG may be of relevance in control of primary

infection *in vivo* and perhaps in the establishment or maintenance of latency.

We thank Dr M. Frank for preparation of sheep red blood cell reagents.

J. COSTA
A. S. RABSON
C. YEE
T. S. TRALKA

Laboratory of Pathology, National Cancer Institute,
National Institutes of Health,
Bethesda, Maryland 20014

Received 2 June; accepted 22 July 1977.

- ¹ Watkins, J. F. *Nature* **202**, 1364 (1964).
- ² Yasuda, J. & Milgrom, F. *Int. Arch. Allergy* **33**, 151 (1968).
- ³ Westmoreland, D. & Watkins, J. J. *gen. Virol.* **24**, 167 (1974).
- ⁴ Costa, J. C. & Rabson, A. S. *Lancet* **i**, 77 (1975).
- ⁵ Lehner, T., Wilton, J. M. A. & Shillito, E. J. *Lancet* **ii**, 60 (1975).
- ⁶ Costa, J., Yee, C., Troost, T. & Rabson, A. *Nature* **252**, 745 (1974).
- ⁷ Reid, T. W. *et al. J. natn. Cancer Inst.* **53**, 347 (1974).
- ⁸ Reynolds, H. Y., Atkinson, J. P., Newball, H. H. & Frank, M. M. *J. Immun.* **114**, 1813 (1975).
- ⁹ Jaffe, E. S., Shevach, E. M., Frank, M. M., Berard, C. W. & Green, J. *New Engl. J. Med.* **290**, 813 (1974).
- ¹⁰ Prahl, J. W. *Biochem. J.* **104**, 647 (1967).
- ¹¹ Huber, C. H., Sundstrom, C., Nilsson, K. & Wigzell, H. *Clin. exp. Immun.* **25**, 367 (1976).

Excretion of feline leukaemia virus by naturally infected pet cats

THE transmission of feline leukaemia virus (FeLV) horizontally among cats has been well established¹⁻³ but the source of infectious virus responsible for this transmission has not been determined. C-type particles resembling FeLV have been seen by electron microscopy in many tissues and body fluids from cats having leukaemia and lymphoma⁴⁻⁷. In reference to potential environmental excretion, particles have been seen in the upper and lower respiratory tract, saliva, urine, gut wall, and peripheral blood. These studies, however, have been limited to electron microscopic visualisation and have not studied levels of infectious FeLV in tissues or fluids which could release virus into the environment. Speculation about the source of infectious virus has included urine, saliva, fleas, and blood (from either scratches, bites, or blood sucking arthropods)^{1,8}.

FeLV is so far the only oncogenic RNA virus that is known to be widely disseminated in man's environment, and several epidemiological studies have attempted to determine if an increased risk of human malignancies occurs for individuals exposed to leukaemic cats⁹⁻¹². Furthermore, the US National Cancer Institute has categorised the FeLV and the feline sarcoma virus as agents of 'moderate risk', presumably because they can grow in human cells and/or demonstrate oncogenicity in experimental conditions in certain non-feline species¹³. It thus seems remarkable that little or no information is available on the sources and amounts of infectious FeLV in excretory materials, especially from those taken from naturally infected pet cats.

To understand better the transmission of this disease and to assess the exposure potential of this virus to human contacts of infected cats we have studied the excretion of FeLV in virus-positive cats.

The animals used in all experiments were naturally infected FeLV-positive healthy pet cats who were identified from two sources: a previously characterised cluster house (four cats)¹⁴, or cats referred by community veterinarians (eight cats). The cats were judged positive based on the indirect immunofluorescence test for virus internal antigens in peripheral blood cells². Six of the animals were domestic short hair, three were Abyssinian, two Burmese, and one Siamese. Eight were males and four were females. All were asymptomatic and seemed healthy.

Plasma was collected in heparinised tubes, placed on ice,

separated, and frozen at -90°C until testing. Saliva was collected using small calcium alginate swabs (Calgiswabs, Inolex, Glenwood, Illinois), diluted in 1.5 ml medium and frozen at -90°C until testing. These swabs absorb a constant volume of saliva and allow quantification of infectious virus (unpublished observations). Urine was collected in sterile containers after manual expression from the bladder. Faecal specimens, in approximately 1-g amounts, were collected using calcium alginate swabs and placed in phosphate-buffered saline and frozen before testing at -90°C . After thawing, the suspension was centrifuged briefly at 1,000 r.p.m. and the supernatant was passed through a 0.22- μm filter (Millipore, Bedford, Massachusetts) before placing on cell cultures. Fleas were individually vacuumed off from cats, pooled in groups of ten, and frozen at -90°C until testing. Before being placed in cell cultures, the fleas were diluted in 1 ml cell culture medium, homogenised with a Teflon pestle, and centrifuged briefly at 1,000 r.p.m.

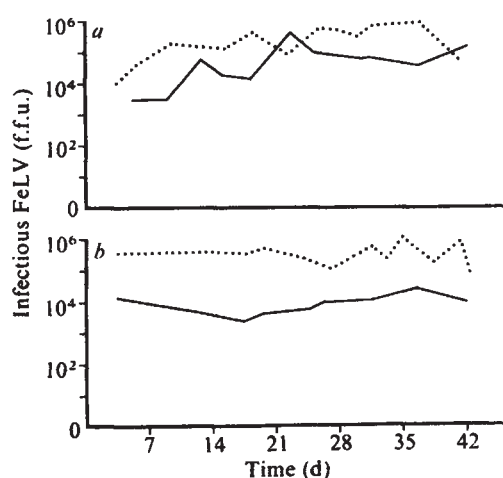


Fig. 1 Simultaneous FeLV titres in plasma (continuous line) and saliva (dotted line) in two cats (a and b) followed for 2 months.

Virus assay was carried out in triplicate using the method of Fischinger¹⁵. Before assay, all specimens were thawed, and tenfold dilutions were made on ice using McCoy's 5a with 15% foetal calf serum containing penicillin 100 IU ml⁻¹, streptomycin 100 μg ml⁻¹, and amphotericin 0.25 μg ml⁻¹.

Simultaneous saliva and plasma samples collected from nine cats were assayed for virus (Table 1). Saliva was positive in all cats examined, containing 5.4×10^3 – 3.9×10^5 focus forming units (f.f.u.) per ml whereas plasma generally contained about fivefold less. Three cats out of the twelve continued to excrete a cytopathic agent in their saliva for the entire length of the study and we were unable to assay their saliva for virus.

Urine was tested from three cats and was negative for infectious virus at the lowest dilution (undiluted or 1:10) (Table 2). Faecal specimens from three cats were tested, and no infectious virus was demonstrated at the

Table 1 Simultaneous saliva and plasma levels of infectious FeLV

Cat	Number of paired specimens*	Saliva f.f.u.	Plasma f.f.u.
1	11	3.9×10^5 †	1.7×10^5 †
2	12	2.4×10^5 †	6.8×10^3 †
3	2	1.6×10^4 †	6.9×10^3 †
4	1	4.4×10^4	3.2×10^4
5	1	3.0×10^5	2.8×10^4
6	1	5.7×10^4	1.6×10^3
7	1	5.4×10^3	1.0×10^3
8	1	1.8×10^4	6.4×10^3
9	1	3.6×10^5	3.2×10^4

*Each specimen was analysed in triplicate.

†Mean.

lowest dilution (0.5 g ml⁻¹) (Table 2). Ten pools of ten fleas each were examined for infectious virus. None was demonstrated at the highest concentration (5 fleas ml⁻¹) (Table 2).

Two cats were followed for 2 months with once or twice weekly FeLV assay for plasma and saliva (Fig. 1). These cats regularly excreted high titres of FeLV in their saliva (10^4 – 10^5 f.f.u. ml⁻¹) and their plasma (10^3 – 10^5 f.f.u. ml⁻¹). Although there was moderate variation of both saliva and plasma, the titres of virus in both remained high and never dropped below 10^3 f.f.u. ml⁻¹. Plasma FeLV alone was assayed in four additional cats for 3 months. The results were similar to those shown in Fig. 1.

Our results indicate that FeLV-infected healthy cats excrete levels of infectious FeLV in saliva that are as high or higher than levels in blood plasma. Although various other tissues and body fluids have revealed virus particles by electron microscopy or serology, our negative results on these suggest that they may be less significant as sources of transmissible virus. In a previous serological study that was non-quantitative, we found detectable levels of FeLV in salivary gland tissue from five out of five leukaemic cats and in urine from three out of five leukaemic cats². Whether cats that are leukaemic excrete infectious FeLV in the same manner as healthy infected cats is unknown. Our study, however, has emphasised non-leukaemic FeLV-infected cats for two reasons: (1) the prevalence of such cats in most random pet populations is considerably higher than the number with leukaemia; and (2) non-leukaemic FeLV-excretor cats have been regularly overlooked or ignored by epidemiologists who examined either cat-to-cat or potential cat-to-man transmission. Such conventional epidemiological studies, especially those attempting to link feline leukaemia to human malignancy, have usually failed to detect horizontal transmission of FeLV or any increased risk of malignancy in human contacts^{12,16}. These investigations, however, chose cases and controls by pathological diagnosis or pet ownership status, and not by virus positivity. We now know that 10–50% of leukaemic cats ('cases') are non-viraemic^{1,2,17}, and that clinically healthy cats or those ill with diseases other than leukaemia or lymphoma ('controls') are often infected with FeLV. Also, serological studies have indicated that many FeLV-infected healthy cats contain detectable plasma virus for several years^{18,19}. In addition, as many as half of the pet cats in free-roaming

Table 2 Infectious FeLV titres in specimens from healthy excretor cats

Specimen	Cats tested	Total	Samples tested (f.f.u.)*	% Positive	Mean titre (f.f.u.)	Range (f.f.u.)
Plasma	12	70	70	100	4.4×10^4	9.4×10^3 – 8.4×10^5
Saliva	9†	30	30	100	2.2×10^5	5.4×10^3 – 2.0×10^6
Urine	3	5	0	0	—	—
Faeces	3	3	0	0	—	—
Fleas	4	10‡	0	0	—	—

*Each tested in triplicate.

†Three cats consistently and four others periodically excreted an agent toxic to our cell cultures.

‡Total of 100 fleas.

populations have evidence of transient FeLV infection, and only small proportions of those that remain chronically infected ultimately develop leukaemia or lymphoma^{1,20}. Therefore, epidemiological studies to date designed to ascertain human risk of FeLV exposure have not precisely done so since virus exposure was probably present in both the case and control groups.

In conclusion, it seems too early to rule out a link between any human disease and exposure to FeLV. Many FeLV-infected cats exist in the community and the laboratory methods to identify them are readily available. Subsequent studies designed to examine potential human risk from FeLV should compare the incidence of human disease in contacts of FeLV-positive cats to matched controls in FeLV-negative cats.

This work was supported by the American Cancer Society and the US National Cancer Institute. D.F. is a Career Development Award recipient from the Center for Disease Control, Atlanta, Georgia, and M.E. and W.D.H. Jr are Scholars of the Leukemia Society of America. We thank Drs S. Cotter and E. Essex for clinical assistance, and D. Gayzagan for technical assistance.

D. P. FRANCIS
M. ESSEX

Department of Microbiology,
Harvard School of Public Health,
Boston, Massachusetts 02115

W. D. HARDY, JR

Memorial Sloan-Kettering Cancer Center,
New York, New York 10021

Received 29 May; accepted 11 July 1977.

- ¹ Essex, M. *Adv. Cancer Res.* **21**, 175 (1975).
- ² Hardy, W. D., Jr *et al. Nature* **244**, 266 (1973).
- ³ Jarrett, W. *et al. J. natn. Cancer Inst.* **51**, 833 (1973).
- ⁴ Dougherty, E., Post, J. E. & Rickard, C. G. *Can. Vet. J.* **10**, 291 (1969).
- ⁵ Gardner, M. B., Rongey, R. W., Johnson, E. Y., DeJournett, R. & Huebner, R. J. *J. natn. Cancer Inst.* **47**, 561 (1971).
- ⁶ Hardy, W. D., Jr *et al. Science* **166**, 1019 (1969).
- ⁷ Laird, H. M., Jarrett, O., Crichton, G. W. & Jarrett, W. F. H. *J. natn. Cancer Inst.* **41**, 867 (1968).
- ⁸ Hardy, W. D., Jr *et al. in Comparative Leukemia Research* (eds Ito, Y. & Dutcher, R. M.) **67** (University of Tokyo Press, Tokyo, 1975).
- ⁹ Schneider, R. *Int. J. Cancer* **10**, 338 (1972).
- ¹⁰ Hanes, B. *et al. J. natn. Cancer Inst.* **45**, 1155 (1970).
- ¹¹ Bross, I. D. J. & Gibson, R. J. *Med. exp. Clin.* **1**, 180 (1970).
- ¹² Essex, M. & Francis, D. P. *J. Am. Animal Hosp. Ass.* **12**, 386 (1976).
- ¹³ *Biologic Safety Manual for Research Involving Oncogenic Viruses*, National Cancer Institute, 3 October, 1974.
- ¹⁴ Essex, M. *et al. J. natn. Cancer Inst.* **54**, 637 (1975).
- ¹⁵ Fischinger, P., Blevins, C., Nomura, S., *J. Virol.* **14**, 177 (1974).
- ¹⁶ Schneider, R. *Int. J. Cancer* **10**, 345 (1972).
- ¹⁷ Jarrett, W. F. H. *Adv. vet. Sci. comp. Med.* **19**, 165 (1975).
- ¹⁸ Essex, M., Hardy, W. D., Jr, Cotter, S. M., Jakowski, R. M. & Sliski, A. *Inf. Immunity* **11**, 470 (1975).
- ¹⁹ Cotter, S. M., Essex, M. & Hardy, W. D. *Jr Cancer Res.* **34**, 1061 (1974).
- ²⁰ Jarrett, W. F. H. *Bibl. Haemat.* **43**, 209 (1975).

Blood group-like activity released by human mammary carcinoma cells in culture

TUMOUR cells synthesise and release antigenic membrane associated components which circulate either in a free state or complexed with host immunoglobulins¹⁻³. A membrane derived N-like glycoprotein, which inhibited *Vicia graminea* lectin haemagglutination, has been demonstrated in serum and ascites fluid of mice bearing the Ha subline of TA₃ murine mammary adenocarcinoma⁴. Springer *et al.*⁷ have shown that M and N blood group reactive substances were present in both benign and malignant lesions of the human breast. But, T-like *Arachis hypogaea* reactive substances were only found in malignant breast tissue and not in benign tumours or healthy breast tissue⁷. Isolation and long term cultivation of a human mammary tumour cell line, BOT-2 was recently reported⁸. Immunological tests indicated that women with diagnosed mammary cancer had circulating antibodies that reacted with these cells⁹ and induced cell surface shedding¹⁰. Here we report the use of blood-typing antisera and lectins to characterise the proteins synthesised and spontaneously

released from BOT-2 cells growing in defined medium. To our knowledge this is the first demonstration of M, N, T, Tn erythrocyte-like glycoproteins being spontaneously shed from human epithelial tumour cells in culture.

BOT-2 cells, after 2 d growth on Eagle's minimum essential medium with 10% foetal calf serum, were grown to confluency (2 weeks) in albumin containing, serum free medium (Hi-WO₅/BA₂₀₀₀; International Scientific). After the 2-week interval, each third day the medium was collected from the culture flasks, centrifuged at 200g for 10 min to remove debris and lyophilised. The dry solids were resuspended in distilled water, dialysed against phosphate buffered saline pH 7.2 (PBS) and chromatographed on Sephadex G-10 equilibrated with PBS. The G-10 excluded proteins were then concentrated to 5 mg ml⁻¹ by pressure dialysis on an Amicon XM-50 membrane. The concentrated protein was tested for reactivity against human blood-typing lectins and antibodies. First the BOT-2 protein was screened for reactivity against various lectins by double diffusion in gel. Then, more sensitive agglutination inhibition assays were performed to show the competition of BOT-2 proteins and M or N red cells for specific anti-M or N antibody binding. Finally because of the potential significance of spontaneously shed glycoproteins with T-like activity to tumour growth and survival, a more sensitive test using shed proteins to inhibit T-erythrocyte agglutination was used.

Double diffusion reactions of BOT-2 proteins and selected blood group typing lectins are shown in Table 1. It may be seen that BOT-2 proteins formed precipitin bands with a variety of lectins having specificities related to blood group antigens M, N, T and Tn. To show cross-reactivity of BOT-2 proteins and the erythrocyte glyco-

Table 1 Blood group active lectins which form precipitin bands with shed human breast tumour cell proteins

Blood group	Lectins	Refs
Activity	Common name	Systematic name
anti-M	Candytuft	<i>Iberis amara</i>
anti-N	—	<i>Vicia graminea</i>
anti-T	Peanut	<i>Arachis hypogaea</i>
anti-Tn	Blue beard	<i>Salvia horminum</i>
anti-Tn	Horse gram	<i>Dolichos biflorus</i>

Seeds were ground dry, mixed with four times their weight of 0.15 M NaCl for 3 h at 20 °C, centrifuged 10 min at 3,000g and the supernatant solution collected. *A. hypogaea* lectin was further purified by affinity chromatography on β -galactosyl derivatives coupled to Sepharose 4B. The lectins (5–20 mg ml⁻¹) were reacted with a 10X concentrate (5 mg ml⁻¹) of dialysed BOT-2 cell growth medium by diffusion tests in 1% Agarose in PBS¹⁶.

proteins by a system involving different reaction mechanisms, a specific antiserum-red cell agglutination inhibition was performed. Agglutination of human M and N erythrocytes by both human and rabbit anti-M and anti-N antisera was inhibited by BOT-2 proteins (Table 2). When a more sensitive assay of T-like activity in BOT-2 proteins was used, the agglutination of human T red blood cells (neuraminidase-treated erythrocytes) by anti-T lectin (*A. hypogaea*) could be completely inhibited by BOT-2 proteins (Table 3). Inhibition required 3,000 times as much BOT-2 proteins as anti-T lectin on a weight basis, however.

In all assays two types of controls were performed. In one, unused medium was concentrated to equal the concentration of medium from BOT-2 cells. In the other, growth medium from a human melanoma cell line was prepared in the same manner as the BOT-2 proteins. When the concentrated unused or melanoma growth medium were tested by double diffusion, antibody agglutination inhibition or lectin agglutination inhibition, no M, N, T or Tn activity was found.

Shed glycoproteins from human breast carcinoma cells

Table 2 Effect of shed human breast tumour cell proteins on the agglutination of M or N erythrocytes by human and rabbit M or N antiserum

Erythrocytes and antiserum	Human antiserum BOT-2 cell proteins (μ g)	Inhibition of agglutination	Erythrocytes and antiserum	Inhibition of agglutination
M	65	Yes	M, 1:2	No
M	32	No	M, 1:4	No
M	16	No	M, 1:8	Yes
			M, 1:16	Yes
N	65	Yes	N, undiluted	No
N	32	No	N, 1:2	Yes
N	16	No	N, 1:4	Yes

Using human antiserum, 50 μ l of serum were incubated with 50 μ l of the indicated amount of BOT-2 cell proteins. Using rabbit antihuman M or N antiserum, 25 μ l of serum at the indicated dilutions were incubated with 25 μ l (32 μ g) of BOT-2 cell proteins. After 15 min incubation at 20 °C, 25 μ l of a 3% suspension of washed human M or N erythrocytes were added to the reactions. After incubation for 90 min, the agglutination reactions were read by visual inspection. The haemagglutination titres of the human M and N antisera were both 1:8. The haemagglutination titres of the rabbit M and N antisera were both 1:64.

in culture react with the same lectins as do the membrane antigens isolated from whole human breast tumour tissue⁷ and the glycoproteins in serum and ascites fluid from mice bearing the TA₃-Ha mammary carcinoma⁸. The finding that BOT-2 glycoproteins reacted with anti-M lectin (*I. amara*) and anti-N lectin (*V. graminea*) suggests that the carbohydrate portions of BOT-2 proteins are similar to that of M and N blood group substances. The reaction of BOT-2 proteins with anti-T (*A. hypogaea*) and anti-Tn (*S. horminum*, *D. biflorus*) may be due to incomplete glycosylation of some of the carbohydrate chains by BOT-2 cells, since T and Tn activity may be induced from N substance by selective enzymatic digestion¹⁶⁻¹⁹.

Other workers have found M and N activity in both benign and malignant breast tumours, but T and Tn activities have been reported to occur only in malignant human breast tumour tissue. A significant number but not all patients with active mammary carcinomas had lowered anti-T serum activity, but after surgical excision of the tumour many of these patients had increased serum anti-T activity²⁰.

Our findings show that a human ductal mammary carcinoma cell line growing in chemically defined media spontaneously releases glycoproteins which have M, N, T and Tn activity. These observations are in accord with the idea that early survival of these cells may depend on presentation of a cell surface similar to M and/or N erythrocyte membrane glycoproteins. As the tumour mass increases it ceases to form fully glycosylated membrane glycoproteins. The resulting incompletely glycosylated M-N precursors (that is, T and Tn) may block or overwhelm the hosts immune capabilities with the result that rapidly growing and aggressive tumour cells have decreased restraint upon survival and growth.

We thank R. J. McNeil, P. J. Riggs, and P. L. Munson for technical assistance; Dr Jim Lawson, Tulsa Regional Red Cross Blood Center, for useful conversations and

the gift of some seeds; Richard Murdock (Ortho Pharmaceuticals) for human M and N antisera; Bill Deal and Twanda Brueggen for typed blood; and the American Cancer Society (BC-230) and the Maizie Wilkonson award for support.

J. H. ANGLIN, JR*

Departments of Biochemistry and Molecular Biology
and Research Dermatology,

MICHAEL P. LERNER

Department of Microbiology and Immunology,

ROBERT E. NORDQUIST

Departments of Anatomical Sciences and
Research Dermatology

University of Oklahoma Health Sciences Center,
Oklahoma City, Oklahoma 73190

Received 13 June; accepted 22 July 1977.

*To whom reprint requests should be addressed.

- Hollinshead, A. C. *et al. Cancer* **34**, 1235-1243 (1974).
- Currie, G. A. & Basham, C. *Br. J. Cancer* **26**, 427-438 (1972).
- Thomson, D. M. P. & Alexander, P. *Br. J. Cancer* **27**, 35-47 (1973).
- Alexander, P. *Cancer Res.* **35**, 2077-2082 (1974).
- Kim, U., Baumler, A., Carruthers, C. & Bielak, K. *Proc. natn. Acad. Sci. U.S.A.* **72**, 1012-1015 (1975).
- Cooper, A. G., Codrington, J. F. & Brown, M. C. *Proc. natn. Acad. Sci. U.S.A.* **71**, 1224-1228 (1974).
- Springer, G. F., Desai, P. R. & Banatwala, I. *J. natn. Cancer Inst.* **54**, 335-339 (1975).
- Nordquist, R. E., Ishmael, D. R., Lovig, C. A., Hyder, D. M. & Hoge, A. F. *Cancer Res.* **35**, 3100-3105 (1975).
- Nordquist, R. E., Schafer, F. B., Manning, N. E., Ishmael, D. R. & Hoge, A. F. *J. Lab. clin. Med.* **89**, 257-261 (1977).
- Nordquist, R. E., Anglin, J. H., Lerner, M. P. *Science* **197**, 366-367 (1977).
- Dahr, W., Uhlenbruck, G. & Bird, G. W. *G. Vox Sang.* **28**, 133-148 (1975).
- Uhlenbruck, G. & Dahr, W. *Vox Sang.* **21**, 338-351 (1971).
- Lotan, R., Skutelsky, E., Danon, D. & Sharon, N. *J. biol. Chem.* **250**, 8518-8523 (1975).
- Bird, G. W. & Wingham, J. *Vox Sang.* **26**, 163-166 (1974).
- Ouchterlony, O. *Acta. Path. Micro. Scand.* **32**, 231-240 (1953).
- Uhlenbruck, G., Pardoe, G. I. & Bird, G. W. *Z. Immunitätsforsch.* **138**, 423-433 (1969).
- Springer, G. F., Tegtmeyer, H. & Huppikar, S. V. *Vox Sang.* **22**, 325-343 (1972).
- Springer, G. F. & Desai, P. R. *Biochem. biophys. Res. Commun.* **61**, 470-475 (1974).
- Springer, G. F. & Desai, P. R. *Carbohydr. Res.* **40**, 183-192 (1975).
- Springer, G. F., Desai, P. R. & Scanlon, E. F. *Cancer* **37**, 169-176 (1976).

A-specific autoantigenic ovarian glycolipids inducing production of 'natural' anti-A antibody

ACCORDING to current concepts, those antibody populations which appear spontaneously in the sera of many species against components of the widely-distributed ABH (blood-group) antigen system are due to stimulation by corresponding exogenous (environmental) antigenic material¹⁻³. We present here evidence which strongly suggests an alternative origin in the mouse.

We recently reported that the development of 'natural' antibodies against structural elements of the common (blood-group) antigen A is markedly sex-dependent in C57BL/10 inbred mice. In these experiments all the sera of

Table 3 Effect of shed human breast tumour cell proteins on the agglutination of human T erythrocytes by purified anti-T lectin

BOT-2 cell proteins (μ g)	Agglutination of T erythrocytes
300	No
150	No
75	Yes
38	Yes

Human T erythrocytes were prepared by neuraminidase treatment of human O erythrocytes⁷. Purified anti-T lectin (*A. hypogaea*) 50 ng was incubated for 15 min at 20 °C with the indicated amount of shed proteins from BOT-2 cells; 0.2 ml of a 3% suspension of T erythrocytes was then added and the mixture incubated for 90 min. The agglutination reaction was determined by visual inspection. The haemagglutination titre of the peanut lectin was 1:10,000.

the females of healthy, unvaccinated mice displayed high levels of antibodies which selectively agglutinate the erythrocytes of the human blood group A, but only about 50% of the sera of the males exerted detectable activities. This pronounced production of 'natural' anti-A antibody in females having a normal oestrous cycle and fertility correlates with the synthesis of ovarian glycolipids specifically combining with the syngeneic 'natural' anti-A antibody, which they strongly inhibit. Such auto-reactivity was not detected in the glycolipid fractions from any of the other male and female murine tissues at statistically significant levels⁴. Moreover, anti-A specific complement-dependent lytic serum activities which discriminate between different (blood-group) A-antigen expressions or qualities and are also exclusively inhibited by the syngeneic ovarian glycolipids could be demonstrated in a further study⁵, and similar observations have been made in limited (unpublished) studies with DBA/2, DDS and NMRI mice.

The genetic background of these findings is as yet unknown, and the site of appearance of the A-active glycolipid structures in the ovarian tissue and the type of cell or cells engaged in their production could up to now also not be determined. But the experiments presented here (Fig. 1) show that these glycolipid structures are probably not expressed at birth but appear during post-natal ovarian maturation and that only low levels of the 'natural' anti-A antibody are produced in the absence of the ovary. At 10 d of age the murine ovarian tissue displays extremely weak A-specific auto-reactivity which then increases rapidly during the maturation process. Marked auto-reactivity can already be measured at the age of 20 d, that is, long before the first ovulation, but complete expression of this reactivity is not reached before the age of 30–40 d and thus correlates with the onset of puberty in the mouse. In fact, no further increase could be measured thereafter. This is illustrated in Fig. 1a, which also shows that the development of the A-specific auto-reactive structures markedly precedes the appearance of the anti-A antibody (Fig. 1b). The antibody develops poorly in mice ovariectomised at the age of 20 d, whereas the development of the antibody levels in sham-operated mice does not significantly differ from that in non-operated mice. Hence, the 'natural' anti-A antibody in the C57BL/10 female mouse is an autoantibody, that is, the syngeneic ovarian glycolipids not only combine with this antibody as a result of, for example, coincidental cross-reactivity, but are also specifically engaged in its induction.

The crucial carbohydrate determinant of these glycolipids involves *N*-acetyl-D-galactosamine in terminal linkage, as the glycolipid material prepared by the method previously described¹ strongly inhibits the agglutinins specifically combining with this amino sugar. Four haemagglutinating units of the anti-A specific lectin from *Dilochos biflorus* (Behringwerke) and the anti-A haemagglutinin from *Helix pomatia* (from Dr E. Cohnen) in volumes of 20 μ l were incubated with equal volumes from twofold serial dilutions of the ovarian glycolipids in 130 mM NaCl–25 mM Na-phosphate buffer, pH 7.2 (PBS), for 60 min at 24 °C. Twenty μ l of a 1% suspension of washed human blood group A₁ erythrocytes in PBS were then added and the incubation was continued for another 60 min. The amounts of the glycolipids producing complete haemagglutination were determined by microscope. The four haemagglutinating units of the *D. biflorus* lectin and the *H. pomatia* haemagglutinin were completely inhibited by 5 and 2.5 μ g of the glycolipid material respectively.

Whatever their other structural properties, which are currently being investigated, such glycolipids are probably not restricted to ovarian tissue. Moreover, the auto-reactive structures discovered in the ovary may also be produced in other murine tissues but in quantities too low to be

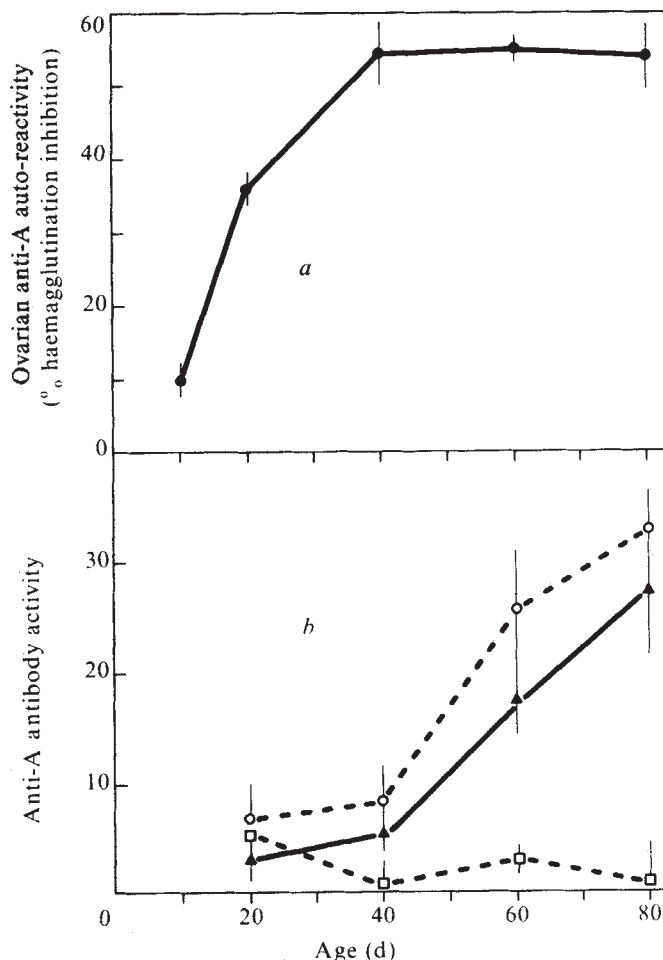


Fig. 1 *a*, Age-dependent appearance of ovarian A-specific auto-reactivity. 10-, 20-, 40-, 60- and 80-d-old healthy, unvaccinated C57BL/10 female mice from our colony (from Jackson Laboratory, Bar Harbor, Maine), each age group consisting of 3 $\times$ 20 animals, were killed by ether inhalation in three separate experiments. Whole venous blood was drawn from the caval veins of the 80-d-old mice, pooled, and the serum to be used in the inhibition experiments 'inactivated' for 15 min at 56 °C. The ovaries were removed and pooled according to the respective groups. The ovarian polar glycolipids were prepared as previously described¹ and dissolved in 130 mM NaCl–25 mM Na-phosphate buffer, pH 7.2, at 100 μ g ml⁻¹. The anti-A-inhibitory activities of the glycolipid material from the ovaries of the different age groups were measured and are given as % inhibition of the haemagglutinating power of the pooled syngeneic serum from the 80-d-old C57BL/10 females for human blood group A₁ erythrocytes according to the previously described quantification procedure¹. Values represent the arithmetic mean and standard deviation obtained from the three different inhibition experiments. *b*, Age-dependent appearance of 'natural' anti-A antibody activities in the sera of unvaccinated C57BL/10 female mice. $\blacktriangle$, Untreated (non-operated) mice; $\square$, mice ovariectomised at the age of 20 d; $\circ$, sham-operated mice. Animals to be ovariectomised or sham-operated were anaesthetised by intraperitoneal injection of 0.1 ml (40 mg per kg body weight) of pentobarbital sodium (Nembutal, C. H. Boehringer). Ovaries were removed by standard techniques. In sham-operations performed in parallel, the peritoneal cavity was opened and closed, but the ovaries were left intact. Three different groups, each consisting of 3 $\times$ 20 ovariectomised and 3 $\times$ 20 sham-operated animals, were killed on the day of the operation and at the age of 40, 60 and 80 d in three separate experiments. Corresponding groups of non-operated animals were killed in parallel. The sera were prepared from blood drawn from the caval veins, pooled according to the respective groups and 'inactivated' for 15 min at 56 °C. The anti-A haemagglutinin activities were determined using human blood group A₁ erythrocytes¹. Each value represents the arithmetic mean and standard deviation obtained from the three different experiments. Values on ordinate derived from⁴

$$\sum_{i=1}^k [\text{score}_i \times (-\log_2 (\text{dilution}) + 1)]$$

demonstrable by the methods used in our previous studies^{4,5}. The low anti-A antibody levels in the sera of the males described in those studies and in the sera of ovariectomised females (see Fig. 1b) could reflect extra-ovarian autoantigenic structures, although the possibility of extraneous stimulation due to environmental A-antigenic components cannot be excluded.

Nevertheless, the data presented here strongly suggest that the production of the 'natural' anti-A antibody in the female mouse originates predominantly from autoimmunisation due to an ovarian autoantigen characterised by the A-specific auto-reactivity discovered. It could be argued that this autoimmunisation is induced by material released from the autoantigenic ovarian structures, but that these structures are probably not available to the antibody *in situ*. In fact, many potentially immunoreactive glycolipids in mammalian cell membranes are effectively shielded by proteinaceous cover and do not react with the antibodies directed against them. This applies particularly to a variety of globosides which display *N*-acetyl-D-galactosamine residues demonstrating predominantly Forssman-type specificity and becoming available to their corresponding antibodies only after protease treatment of the cell surface^{6,7}. Although the murine ovarian A-like autoantigen does not seem to involve this particular 'classic' heterophile specificity, its components might also well be 'masked' *in situ* and protected from immunological attack in normal conditions. Thus the functional significance of the findings reported is still not known. Moreover, the intriguing question of whether these findings may be extrapolated to other species and whether the majority of human 'natural' anti-A (iso)antibody populations may also basically reflect growth processes requires further investigation.

We thank Miss Maria Charlier and Miss Anneliese Rosenbaum for technical assistance and Mrs Beverly B. Niemann for editorial assistance.

P. AREND
J. NIJSSEN

Department of Chemotherapy,
Department of Biometrics,
Research Laboratories,
Chemie Grünenthal,
Postfach 129,
Zweifaller Strasse 112,
D-5190 Stolberg/Rhld, FRG

Received 3 May; accepted 19 July 1977.

¹ Springer, G. F., Horton, R. E. & Forbes, M. J. *exp. Med.* 110, 221-244 (1959).
² Springer, G. F., Williamson, P. & Brandis, W. C. *J. exp. Med.* 113, 1077-1093 (1961).

³ Springer, G. F. & Horton, R. E. *J. clin. Invest.* 48, 1280-1291 (1969).

⁴ Arend, P. & Nijssen, J. *J. Immunogenet.* 3, 373-382 (1976).

⁵ Arend, P. & Nijssen, J. *Z. Immun. Forsch.* 153, 74-84 (1977).

⁶ Uhlenbruck, G., Reifenberg, U. & Heggen, M. *Z. Immun. Forsch.* 139, 486-499 (1970).

⁷ Burger, M. M. *Nature new Biol.* 231, 125-126 (1971).

Reconstruction of photoreceptor membrane potentials from simultaneous intracellular and extracellular recordings

INSERTING a microelectrode into a cell to record electrical events provides a rapid and direct way of investigating many fundamental membrane-based processes^{1,2}. The intracellular signals so obtained do not, however, represent a true picture of the underlying permeability changes if significantly large extracellular potentials are also present. As this is indeed the case in a wide range of tissues, including the cephalopod retina, we have recorded simultaneously the intracellular (V_i) and extracellular (V_e) signals from the photoreceptors of *Sepiolo atlantica* in order to reconstruct the true transmembrane potential (V_m). We show here that the discrepancies between

V_i and V_m depend both on the magnitude and duration of the light stimulus.

Extracellular potentials of the order of 30 mV have been recorded from arthropod^{3,4} and cephalopod retinas (R. B. Clark and G.D., unpublished). These are set up by currents passing through regions of high resistance in the restricted space between adjacent photoreceptor cells (Fig. 1), and several equivalent circuit models that take account of this have been put forward⁴⁻⁶. One implication of these models, which has often been overlooked, is that, when the potential at the back of the eye is used as a reference point, the intracellular recording (V_i) will not give a true picture of the actual photoreceptor membrane potential change (V_m), but will in fact measure V_m in series with V_e (Fig. 1). As most investigators of both invertebrate and vertebrate retinas use this recording arrangement (see refs 1 and 2 for reviews), we felt it worthwhile to find out whether there are significant differences between observed V_i and computed V_m potentials (Fig. 1).

We have found that the most stable intracellular recordings are obtained from the cephalopod when the electrode enters a cell almost immediately after touching the distal surface of the retina. Before entering a cell, we first ensured that the extracellular signals from both recording

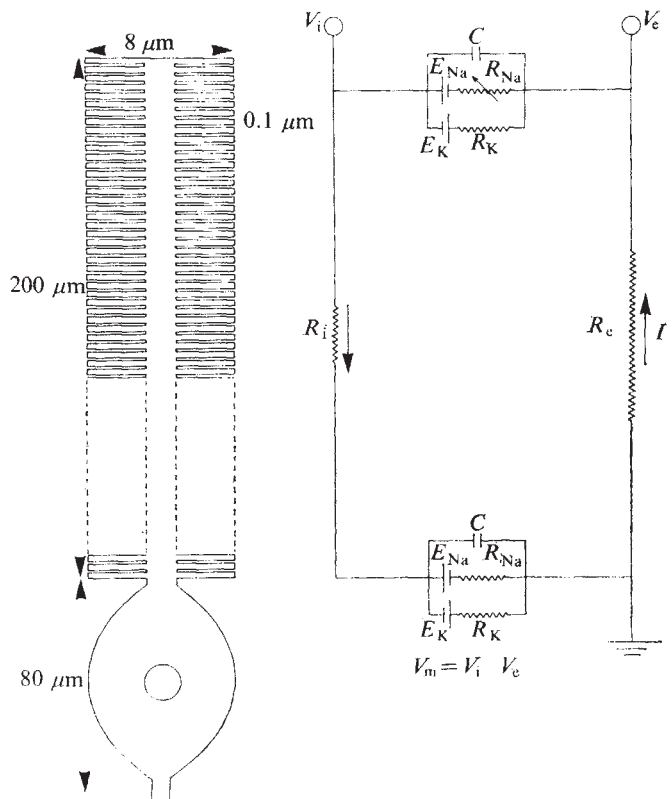


Fig. 1 Diagrammatic representation of a cephalopod photoreceptor cell based on the electron micrographs of refs 18 and 19. There is a minimal extracellular space between cells, whereas in the vertebrate retina the rods and cones are smaller and more widely spaced²⁰. The equivalent circuit is essentially the same cephalopod model as in ref. 6 and resembles an arthropod model⁴. The sodium resistance (R_{Na}) in the distal segment is assumed to decrease in the light. Photocurrents (I) flowing across a large extracellular resistance (R_e) imply a correspondingly large V_e . R_K is usually assumed constant and R_i small, but the validity of neither of these assumptions affects our basic argument which is that the intracellular signal (V_i) does not give a true measure of the primary transmembrane voltage change (V_m). In the recording arrangement used here with the reference electrode (in this case a silver/silver chloride wire loop) at the back of the retina, V_m is given by the difference between V_i and V_e . As V_i and V_e have opposite signs, this means in effect adding the amplitudes of the signals in order to reconstruct V_m .

electrodes were identical. The fact that we could do this with electrodes that were spaced at least 1 mm apart showed the homogeneity of the illumination conditions and, more importantly, the homogeneity of radial current flow across the retina.

Simultaneous extracellular and intracellular records were obtained in response to both short and long flashes from the non-perfused retina maintained at 10 °C (see ref. 7 for experimental details). The short flashes from a stroboscope were 10 μ s in duration and so were well within the latency period of the response at all intensities (Fig. 2). At low intensities ($\log I = -3.2$ and below), the time courses of the two signals are similar whereas the amplitude of the intracellular is the larger. Thus, over this intensity range, the intracellular signals are representative of the true transmembrane potential changes. At higher intensities, the two responses have different time courses and are of comparable magnitude. They are complementary in that they sum algebraically to give a flat region of maximal amplitude. This becomes more apparent as the intensity increases.

The recovery to the baseline is complete within 500 ms at low intensities, whereas there is a significant delay in recovery at the higher intensities. This after-depolarisation, which has been observed in a number of invertebrate preparations^{8,9}, increases with increasing intensity. In vertebrate rods and cones there is an after-hyperpolarisation which increases with intensity¹⁰⁻¹². At the highest intensity used in the present study ($\log I = 0$) there is a very pronounced transient phase in the intra-

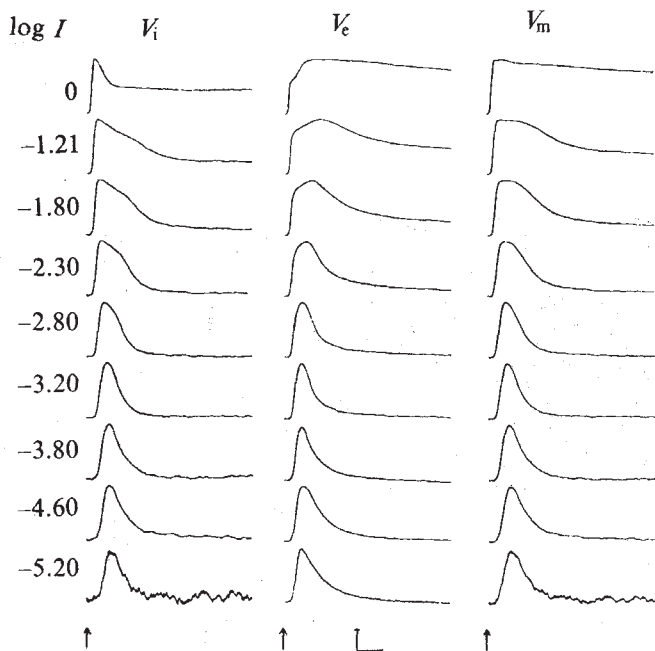


Fig. 2 Response to short flashes. Simultaneous recordings of intracellular and extracellular photoreceptor potentials of the cephalopod retina. The beginning of each trace (arrowed) corresponds to the arrival of a 10- μ s xenon tube flash of unfiltered incident energy equal to 10^{-2} J m $^{-2}$ of white light ($\log I = 0$). The relative intensity of each flash is given on the left of the figure. The resting potential was -45 mV in all of the intracellular recordings (depolarising upwards). The dark potential in the extracellular recordings was zero and an upward deflection in this case corresponds to a negative-going potential at the microelectrode. The signals were digitised, filtered above 1 kHz and plotted. The horizontal scale bar represents 150 ms and the vertical bar represents mV, which for the various intensities is given (in the sequence V_i , V_e , V_m) as follows: $\log I = -5.2$: 0.4, 0.3, 0.7; $\log I = -4.6$: 1.5, 1.0, 2.4; $\log I = -3.8$: 3.6, 2.8, 5.9; $\log I = -3.2$: 6.8, 5.7, 12.1; $\log I = -2.8$: 8.2, 7.6, 15.6; $\log I = -2.3$: 8.8, 9.1, 17.4; $\log I = -1.8$: 9.2, 9.6, 17.7; $\log I = -1.21$: 9.5, 9.7, 17.4; $\log I = 0$: 9.4, 11.1, 16.6.

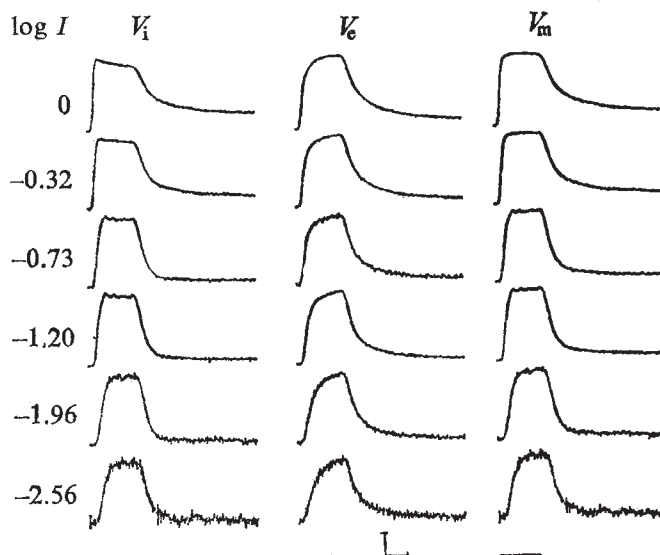


Fig. 3 Response to long flashes. Responses to 500-ms flashes from a light-emitting diode (Plessey GPL5). The light from the diode had an unfiltered energy equal to 0.02 W m $^{-2}$ ($\log I = 0$). The start of each trace corresponds to the stimulus onset which had virtually zero increase-time. The stimulus duration is marked by the solid line below each column. The intracellular dark-adapted potential was -42 mV in each case and the extracellular dark potential was zero. The horizontal scale bar represents 250 ms and the vertical scale bar (mV) for each of the light intensities is given (in the sequence V_i , V_e , V_m) as follows: $\log I = -2.56$: 0.6, 0.2, 0.8; $\log I = -1.96$: 1.3, 0.4, 1.7; $\log I = -1.2$: 3.4, 1.3, 4.7; $\log I = -0.73$: 5.5, 2.3, 7.4; $\log I = -0.32$: 6.7, 3.1, 9.4; $\log I = 0$: 6.8, 3.9, 10.1.

cellular recording which corresponds very closely in time course to the fast response in the extracellular recordings (R. B. Clark and G.D., unpublished). An initial transient has been observed in *Limulus* photoreceptors when short intense flashes were used^{13,14}, and it has been suggested that the transient phase arises from a different mechanism from the remainder of the response¹⁴. It is interesting that the initial transient of V_i in the cephalopod almost completely disappears in the reconstructed membrane response (Fig. 2).

A light-emitting diode was used to deliver 500-ms flashes and in this case the waveforms of the intracellular and extracellular responses appear quite different (Fig. 3). The V_i records are much more rectangular in appearance, and the amplitudes of the responses of V_i were consistently greater than the corresponding V_e records. On closer examination, however, certain similarities between the two types of records emerge. The latencies of both the intracellular and extracellular signals at any one intensity are equal and decrease with increasing intensity, and the initial rate of increase of both signals increases with increasing intensity. At high intensities the intracellular response reaches a maximum value and then decays towards the baseline while the stimulus is still on. This 'sag phase' has also been observed in vertebrate rods and cones¹⁰⁻¹². In the cephalopod, it disappears in the reconstructed membrane responses (Fig. 3). The intracellular records again show a maintained after-depolarisation, which is relatively greater in magnitude than the offset in the V_e records. At high light intensities, as in the short flash responses (Fig. 2), the intracellular and extracellular records sum algebraically to give a flat response over a region in time where both V_i and V_e are changing.

Large extracellular responses are not unique to the invertebrate retina as vertebrate retinal neurones, principally Müller cells¹⁵, can in certain circumstances contribute several millivolts to the overall electroretinogram.

Some workers in this field do take precautions to prevent contamination of primary intracellular photoreceptor recordings by extracellular potentials¹². Extracellular potentials of comparable magnitude are also found, for example, in vertebrate cardiac muscle¹⁶ and olfactory tissue¹⁷. If the membrane events in these tissues are to be analysed in detail, for example in terms of current carriers and permeability changes, then it is important to know the basic time course of the potential change across the receptor membrane. The data presented here strongly indicate that both the intracellular and extracellular signals have to be recorded simultaneously in order to obtain this information. It is also true that in such tissues where the extracellular signal is a function of position (ref. 7 and R. B. Clark and G.D., unpublished), there is no single, true transmembrane potential, but in fact a continuum of transmembrane potentials that require simultaneous recording of the signals from an intracellular electrode and several extracellular electrodes placed at different depths in the tissue. Reconstructions of the primary photoreceptor membrane events based on a simple equivalent model such as that described in Fig. 1 and also a more complex model that takes account of the depth variation of the extracellular signal, will be the subject of a future communication.

We thank Professor E. Rojas and Dr P. C. Croghan for discussions, and Professor P. Fatt of University College for useful criticisms of the manuscript. P.B.P. acknowledges assistance in the form of a scholarship from the University of East Anglia.

P. B. PYNSENT
G. DUNCAN

Biophysics Section,
School of Biological Sciences,
University of East Anglia,
Norwich, UK

Received 12 May; accepted 21 July 1977.

- 1 Tomita, T. *Q. Rev. Biophys.* 3, 179–221 (1970).
- 2 Rodieck, R. W. *The Vertebrate Retina: Principles of Structure and Function* (Freeman, San Francisco, 1973).
- 3 Burt, E. T. & Catton, W. T. *J. Insect Physiol.* 10, 865–886 (1964).
- 4 Shaw, S. R. *Nature* 255, 480–483 (1975).
- 5 Hagins, W. A. *Cold Spring Harb. Symp. quant. Biol.* 30, 403–415 (1965).
- 6 Duncan, G. & Croghan, P. C. in *Biochemistry and Physiology of Visual Pigments* (ed. Langer, H.) 229–233 (Springer, Berlin, 1973).
- 7 Clark, R. B. *Expl Eye Res.* 20, 499–504 (1975).
- 8 Hochstein, S., Minke, B. & Hillman, P. *J. gen. Physiol.* 62, 105–128 (1973).
- 9 Minke, B., Wu, C.-F. & Pak, W. L. *Nature* 258, 84–87 (1975).
- 10 Baylor, D. A., Hodgkin, A. L. & Lamb, T. D. *J. Physiol., Lond.* 242, 685–727 (1974).
- 11 Grabowski, S. R. & Pak, W. L. *J. Physiol., Lond.* 247, 363–391 (1975).
- 12 Coles, J. A. & Yamane, S. *J. Physiol., Lond.* 247, 189–207 (1975).
- 13 Wulff, V. J. & Mueller, W. *J. Vision Res.* 13, 661–671 (1973).
- 14 Wulff, V. J. & Mendez, C. *Vision Res.* 13, 2327–2333 (1973).
- 15 Miller, R. F. & Dowling, J. E. *J. Neurophysiol.* 33, 323–341 (1970).
- 16 Jack, J. B., Noble, D. & Tsien, R. W. *Electric Current Flow in Excitable Cells* (Clarendon, Oxford, 1975).
- 17 Ottoson, D. in *Handbook of Sensory Physiology* 4, 95–131 (Springer, Berlin, 1971).
- 18 Zonana, H. V. *Bull. Johns Hopkins Hosp.* 109, 185–205 (1961).
- 19 Cohen, A. I. *J. comp. Neurol.* 147, 351–378 (1973).
- 20 Penn, R. D. & Hagins, W. A. *Biophys. J.* 12, 1073–1094 (1972).

Immunological identification of rat neurophysin precursors

NEURONES in the supraoptic and paraventricular nuclei of the hypothalamus synthesise two peptide hormones, vasopressin and oxytocin. These hormones and their neurophysin 'carrier' proteins are stored in (and released from) nerve endings in the posterior lobe of the pituitary^{1–3}. On the basis of extensive studies of the synthesis of vasopressin *in vivo* and *in vitro*, Sachs *et al.*^{4–8} have suggested that vasopressin and its associated neurophysin are synthesised as parts of a common precursor by a ribosomal mechanism. We have also presented evidence that the neurophysins are synthesised as parts of larger precursor molecules^{9,10}. These 20,000 molecular weight precursors seem first to give rise to 17,000 molecular weight intermediates which, in turn, yield the 12,000 molecular weight neurophysins. The following data show that the putative neurophysin pre-

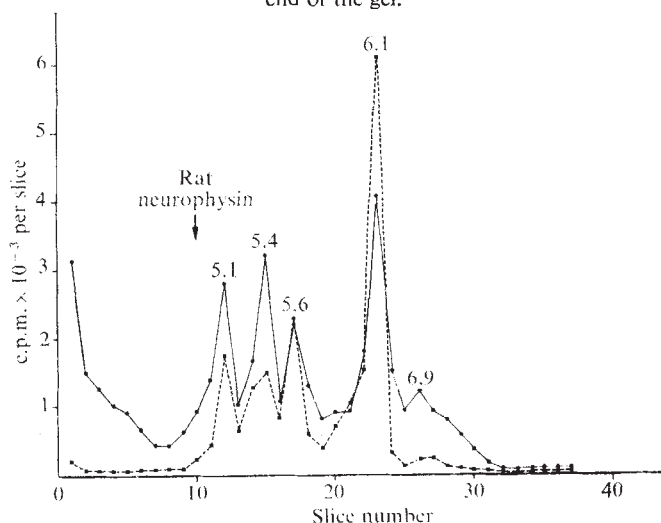
cursors and intermediates bind specifically to anti-rat neurophysin antiserum.

Female Osborne-Mendel rats (250 g) were used. For 5–7 d before use, the rats were given 2% saline to drink to stimulate neurophysin and vasopressin biosynthesis¹⁰. They were then anaesthetised with ether and their heads were fixed in a stereotaxic device. The tips of two 30-gauge needles were positioned just adjacent to the two supraoptic nuclei^{9,10} and 20 μ Ci ³⁵S-cysteine (New England Nuclear; specific activity 40–50 Ci mmol⁻¹) in 1 μ l normal saline containing 1 μ g dithiothreitol were injected through each needle over a 5-min period. The rats were decapitated after an additional 55 min and their brains were quickly removed, mounted on specimen holders, and frozen on dry ice. The brains were placed in a cryostat (–9 °C) and 300 μ m thick frontal sections through the anterior hypothalamus were cut with a microtome. The supraoptic nuclei were dissected from the frozen sections with hollow, stainless steel needles¹¹. The tissue was immediately homogenised in 0.1 M HCl. Tissue pellets from ten animals were pooled into one sample, the final volume of which was 100 μ l. This sample was centrifuged at 10,000g for 5 min, and 10 μ l of the supernatant were subjected to isoelectric focusing in polyacrylamide gels (Fig. 1, solid line)^{10,12}. Four major peaks of labelled protein were present in the supernatant. These had isoelectric points (pI) of 5.1, 5.4, 5.6, and 6.1. A fifth peak (pI 6.9) was also consistently found, but it had less radioactivity than the others. We have previously reported that the pI 5.4 and 6.1 'precursor' proteins—the first ones to be labelled in the pulse-chase paradigm—have molecular weights of about 20,000 and that the pI 5.1 and 5.6 'intermediates' have molecular weights of about 17,000.

The remaining 80 μ l of the supernatant were neutralised with an equal volume of 0.1 M NaOH. An additional 200 μ l of 'buffer A' (0.01 M potassium phosphate, 1.5 M sodium chloride, 1% sodium azide, pH 7.4) were added plus 100 μ l of normal rabbit serum and 0.5 mg of bovine serum albumin. After 4 d at 4 °C the solution was passed over a calibrated Sephadex G-100 column (0.6 $\times$ 17.5 cm) which had been equilibrated with buffer A. Some radioactive material which must either have been of large molecular weight or bound to large serum components appeared in the void volume of the column (Fig. 2a). Analysis of the radioactive substances in the void volume by isoelectric focusing indicated that relatively little of it was 'precursor' or 'intermediate'.

A major peak of labelled material eluted between 2.6 and

Fig. 1 Isoelectric focusing of ³⁵S-labelled proteins in an acid extract of the supraoptic nucleus 1 h after injection of ³⁵S-cysteine adjacent to this nucleus (solid line), and of labelled proteins in the above extract bound by anti-rat neurophysin (broken line). Six per cent polyacrylamide gels were used for isoelectric focusing; the sample buffer contained 8 M urea, 1% Triton X-100, and 2% ampholytes (pH 3–10); and the sample was loaded at the anodal end of the gel.



4.0 ml. Another very large peak came off the column after the bed volume (5 ml), and probably contained cysteine and peptides. A sample containing the material which eluted from the column between 2.9 and 3.5 ml (the '20,000 molecular weight peak') was divided into three 200- μ l aliquots. A specific and well characterised rabbit anti-rat neurophysin (20 μ l)¹³ was added to one of these plus 250 μ l of buffer A; 20 μ l of anti-rat neurophysin plus 20 μ g of rat neurophysin (prepared as described previously¹³) in 250 μ l of buffer A were added to the second; and 20 μ l of normal rabbit serum were added to the

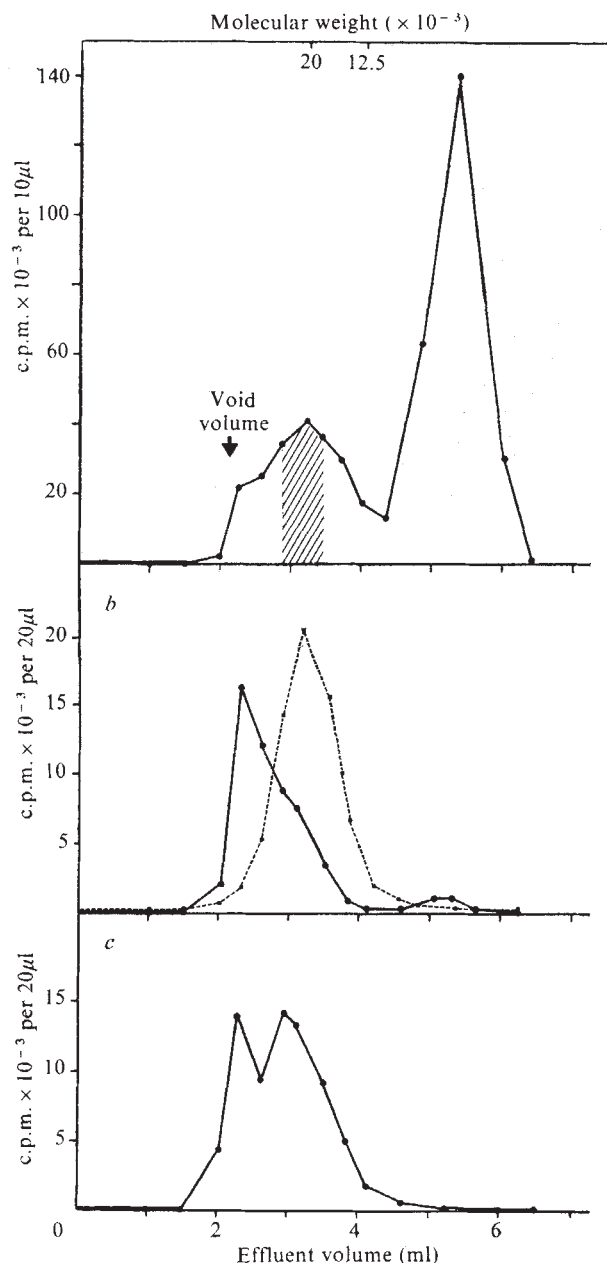


Fig. 2 Sephadex G-100 chromatography of ³⁵S-labelled material extracted from the supraoptic nucleus 1 h after a pulse of ³⁵S-cysteine. *a*, Separation of material which binds non-specifically to large molecular weight components of normal rabbit serum. Large, labelled molecules and smaller molecules bound to large species are found in the void volume. 'Precursors' and 'intermediates' are present in an included volume peak, the middle of which (hatched area) was used for subsequent studies. *b*, Most of the material in the included ('20,000 molecular weight') peak is excluded from the column after addition of rabbit anti-rat neurophysin (solid line). Addition of normal rabbit serum (broken line) caused no change in the elution pattern of the '20,000 molecular weight peak'. *c*, In the presence of rat neurophysin, some labelled protein in the '20,000 molecular weight peak' is excluded from the column (bound to antibody) and part is not.

third. Four days later the three samples were passed in turn over the G-100 column. Few counts appeared in the void volume of the column when normal rabbit serum was added to the '20,000 molecular weight peak'. The labelled material eluted where it had earlier (Fig. 2b), and less than 5% of it could be precipitated by adding goat anti-rabbit IgG. On the other hand, about 75% of the labelled material was found in the void volume of the column when anti-rat neurophysin was added to a sample of the '20,000 molecular weight peak' (Fig. 2b). The labelled species in the void volume could be precipitated in this case by goat anti-rabbit IgG, showing that they were attached to antibody. The antibody-bound materials that eluted between 2.0 and 2.3 ml were precipitated with trichloroacetic acid and subjected to isoelectric focusing. Four peaks were found with isoelectric points corresponding to those of the 20,000 molecular weight precursors ($pI = 5.4 + 6.1$) and the 17,000 molecular weight intermediates ($pI = 5.1 + 5.6$) (Fig. 1, broken line). The pI 6.1 protein seemed to be bound preferentially; the reason for this is unclear. A small amount of pI 6.9 protein may have been bound by the antibody; none of the other components of the original homogenate appeared to be bound.

The counts in the sample of '20,000 molecular weight peak', to which both anti-rat neurophysin and rat neurophysin were added, were distributed between a void volume peak and a 20,000 molecular weight included volume peak (Fig. 2c). The counts in the former peak were precipitated by anti-IgG; the counts in the latter were not; the neurophysin seems to have displaced labelled proteins from the antibody. The displacement may, however, have been incomplete because of (1) the presence of an excess of antibody relative to the amount of neurophysin added or (2) the relatively high affinity of the antibody for the precursors as opposed to neurophysin.

We have shown that, in addition to binding neurophysins¹³, anti-rat neurophysin antiserum binds four larger cysteine-rich proteins found in the supraoptic nucleus. These proteins, which do not react with immunoglobulins in normal rabbit serum to a significant extent, can be displaced from anti-neurophysin by neurophysin itself. These data lend credence to our suggestion that larger protein species synthesised in the supraoptic nucleus give rise to the neurophysins^{9,10}.

We have also studied the synthesis of neurophysin in a strain of rats with diabetes insipidus, that is, the Brattleboro strain¹⁴. These animals do not make vasopressin or its associated neurophysin. They lack the pI 4.8 neurophysin as well as the pI 6.1 precursor and the pI 5.6 intermediate. Thus, the vasopressin-associated neurophysin seems to be made from the pI 6.1 precursor by way of the pI 5.6 intermediate, and the oxytocin-associated neurophysin seems to be made from the pI 5.4 precursor by way of the pI 5.1 intermediate. Whether the precursors or intermediates are glycoproteins as are those of ACTH¹⁵ remains to be determined. Whether the precursors contain oxytocin and vasopressin as well as the neurophysins can now be tested directly.

A.G.R. is supported by the NIG (grant no. AM 16166) and is the recipient of an NIH Career Development Award (grant no. AM 00093). Reprint requests to M.J.R.

MICHAEL J. BROWNSTEIN

Section on Pharmacology,
Laboratory of Clinical Science,
National Institute of Mental Health,
Bethesda, Maryland 20014

ALAN G. ROBINSON

Department of Medicine,
University of Pittsburgh,
Pittsburgh, Pennsylvania 15261

HAROLD GAINER

Behavioral Biology Branch,
National Institute of Child Health and Human Development,
Bethesda, Maryland 20014

Received 25 April; accepted 18 July 1977.

- ¹ Bargman, W. in *Neurophysiological Hormones and Similar Polypeptides* (ed. Berde, B.) 1-39 (Springer, Berlin, 1968).
- ² Heller, H. in *Handbook of Physiology*, section 7: Endocrinology, 4 (ed. Knobil, E., & Sawyer, W. H.) 103-117 (American Physiological Society, Washington, DC, 1974).
- ³ Sachs, H. *J. Neurochem.* 5, 297-303 (1960).
- ⁴ Sachs, H. & Takabatake, Y. *Endocrinology* 75, 943-948 (1964).
- ⁵ Sachs, H., Fawcett, P., Takabatake, Y., & Portanova, R. *Recent Progr. Horm. Res.* 25, 447-491 (1969).
- ⁶ Sachs, H., Pearson, D., & Nureddin, A. *Ann. N.Y. Acad. Sci.* 248, 36-45 (1975).
- ⁷ Takabatake, Y. & Sachs, H. *Endocrinology* 75, 934-942 (1964).
- ⁸ Gainer, H., Sarne, Y., & Brownstein, M. J. *Science* 195, 1354-1356 (1977).
- ⁹ Gainer, H., Sarne, Y., & Brownstein, M. J. *J. Cell Biol.* 73, 366-381 (1977).
- ¹⁰ Palkovits, M. *Brain Res.* 59, 449-450 (1973).
- ¹¹ O'Farrell, P. H. *J. Biol. Chem.* 250, 4007-4021 (1975).
- ¹² Seif, S. M., Huellmantel, A. B., Platia, M. P., Haluszczak, C., & Robinson, A. G. *Endocrinology* 100, 1317-1326 (1977).
- ¹³ Brownstein, M. J. & Gainer, H. *Proc. natn. Acad. Sci. U.S.A.* (in press).
- ¹⁴ Mains, R. E. & Eipper, B. A. *J. Biol. Chem.* 251, 4115-4120 (1976).

Several of the adenosine deaminase isozymes are glycoproteins

THE basis for the complex multiple molecular forms (isozymes) of the enzyme adenosine deaminase (ADA) has been widely studied. The ADA found in human erythrocytes (so-called 'red cell' ADA) exhibits genetically determined polymorphism detectable by electrophoresis¹, has reactive thiol groups² and has a low molecular size³. 'Red cell' ADA is present in various tissues other than the red cell³, but additional adenosine deaminases, the so-called tissue ADA isozymes *a*, *b*, *c*, *d* and *e* named in order of decreasing electrophoretic mobility, which exhibit rather different properties, occur in varying amounts in nonerythroid tissues. With the discovery that the deficiency of 'red cell' ADA in certain patients with combined immune deficiency disease is accompanied by the deficiency of all the other isozymes⁴⁻⁶, it became clear that all the forms were probably coded by a single ADA locus. The 'red cell' ADA can be converted into the characteristic 'tissue' isozymes by tissue extracts both from normal individuals^{7,8} and from patients with combined immune deficiency disease. A converting factor has been isolated and partially purified and is thought to be a protein⁹. This protein is presumably associated with one or several 'red cell' ADA molecules to form the 'tissue' isozymes, accounting for the alteration in properties (increase in molecular size, loss of detectable genetically determined electrophoretic variation and loss of thiol reactivity³). We present here evidence, from lectin affinity chromatography and experiments with neuraminidase, that most of the multiple forms of 'tissue' ADA are glycoproteins which differ in their accessible sugar residues. This suggests that the converting factor may be a single glycoprotein with heterogeneous carbohydrate content.

Treatment of extracts of various human tissues with the enzyme

neuraminidase (sialidase) seems to result in the conversion of the most anodal tissue ADA isozymes (*a*, *b* and *c*) into a zone of activity (*d*₂) which is slightly less anodal than the major *d* isozyme (now referred to as *d*₁). The effects of neuraminidase are most easily demonstrated using extracts of liver (Fig. 1) which contain virtually no 'red cell' ADA. The final pattern resembles that seen in kidney extracts. These changes are presumably due to the removal of negatively charged sialic residues from the tissue ADA *a*, *b* and *c* isozymes. The *d*₁ and *d*₂ isozymes were not affected by treatment with neuraminidase and this is most easily demonstrated using extracts of kidney (Fig. 1). The 'red cell' ADA and the *e* isozyme were also not affected by the neuraminidase treatment.

The ADA isozymes in liver, kidney, intestine and various other tissues were investigated by affinity chromatography on concanavalin A-Sepharose and wheat germ lectin-Sepharose. Concanavalin A is known to bind to glycoproteins containing terminal α -D-mannopyranosyl and α -D-glucopyranosyl residues and internal 2-O-D-mannopyranosyl groups^{10,11}. Wheat germ lectin binds *N*-acetylglucosamine residues specifically and sialic acid residues nonspecifically¹².

'Red cell' ADA and the tissue *e* isozyme failed to bind to either lectin, but the other tissue isozymes showed affinity for one or both lectins. Isozyme *a* was adsorbed by both lectins. The relatively weakly active isozymes *b* and *c* seemed to be adsorbed to concanavalin A but their affinity towards wheat germ lectin was not thoroughly investigated. The *d* isozyme showed heterogeneity in its adsorption characteristics. Re-chromatography experiments showed that this was not due to overloading. In the kidney the more cathodal component (*d*₂) was adsorbed by both lectins whereas the more anodal component (*d*₁) was only partly adsorbed. In the liver and intestine where the major component of the *d* isozyme has the mobility of *d*₁, contrasting results were obtained with wheat germ lectin and concanavalin A. The *d*₁ isozyme was mostly adsorbed by concanavalin A but not by wheat germ lectin (Fig. 2).

Lectin affinity chromatography was also conducted using neuraminidase treated liver extracts since the removal of sialic acid residues might alter the affinity characteristics of the isozymes. The affinity of the isozymes towards concanavalin A was unaffected by neuraminidase treatment, but the results of the experiments using wheat germ lectin were hard to interpret and two-stage experiments were therefore conducted. Untreated liver extracts were first of all fractionated by chromatography on wheat germ lectin-Sepharose. Both the fractions were then treated with neuraminidase. The nonadsorbed *d*₁ isozyme was not affected by this treatment. The adsorbed *a* isozyme was converted into the two forms of the *d* isozyme, *d*₁ and *d*₂ (Fig. 2). This material was then re-chromatographed on wheat germ lectin. The *d* isozyme was largely not adsorbed whereas the *d*₂ isozyme was adsorbed. Thus, it seems that the *a* isozyme exists in at least two forms with overlapping electrophoretic mobilities: one *a*₂ which may contain

Fig. 1 Effect of neuraminidase treatment on the ADA isozyme pattern of red cell, liver and kidney extracts. **A**, Photograph of a starch gel and **B**, a diagrammatic representation of the isozymes. Open rectangles represent the 'tissue' ADA isozymes. Shaded rectangles represent the 'red cell' ADA isozymes. On the gel shown the *d*₁ and *d*₂ isozymes were not clearly resolved, but are illustrated as two isozymes in the diagram. Neuraminidase treatment was performed by incubating 50 μ l of aqueous tissue extract with 20 μ l of neuraminidase solution (from *Clostridium perfringens*, Sigma type VI; 1 unit per ml in 10 mM phosphate, citrate buffer pH 5.0) for 2 h. [Similar results were also obtained using *Vibrio cholerae* neuraminidase.] Electrophoresis and detection were done as described by Edwards *et al.*³.

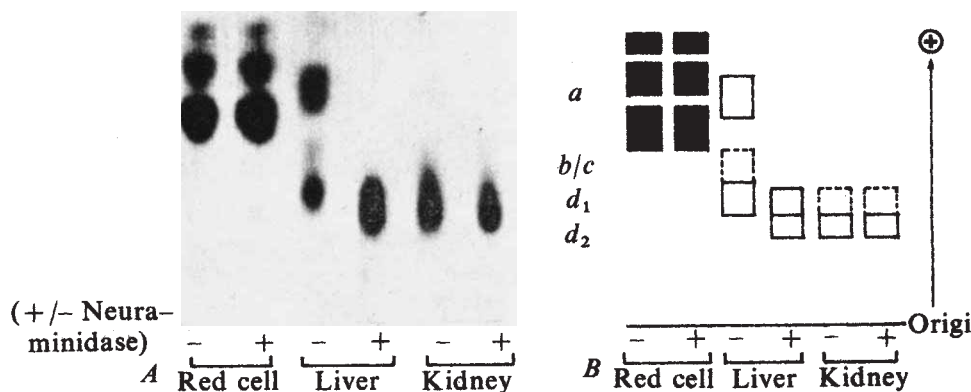
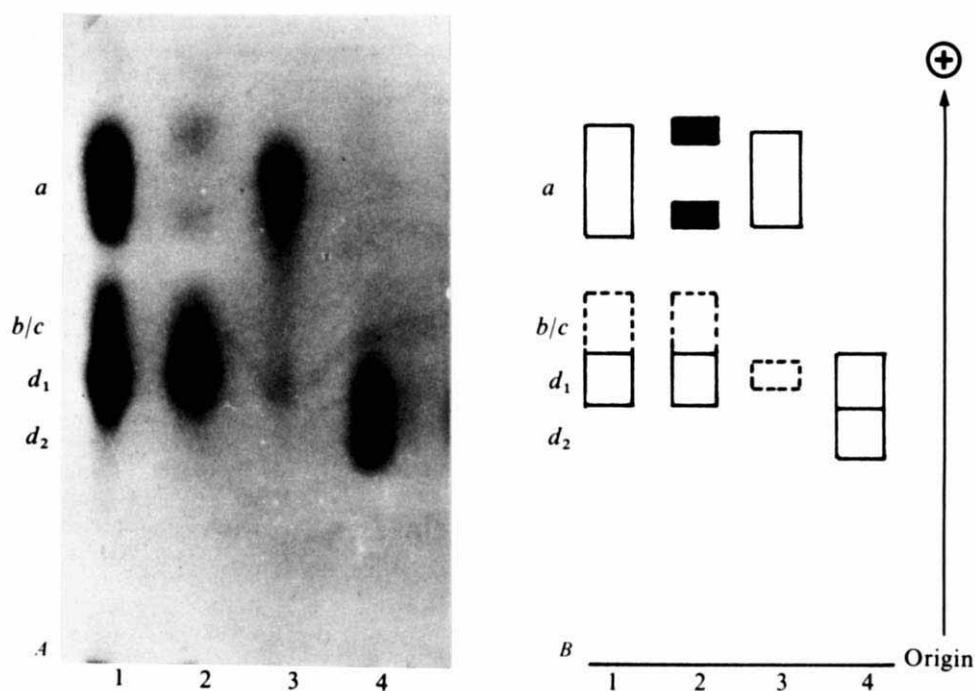


Fig. 2 Affinity chromatography of liver extracts using wheat germ lectin-Sepharose. **A**, Photograph and **B**, diagrammatic representation. 1, Unfractionated control; 2, material that was not adsorbed; 3, material that was adsorbed and subsequently eluted with 10% *N*-acetylglucosamine; 4, sample 3 treated with neuraminidase. The chromatography was conducted using Wheat germ lectin-Sepharose 6MB (Pharmacia) or wheat germ lectin-Sepharose synthesised using Sepharose 4B CNBr-activated (Pharmacia) and wheat germ lectin (Sigma), at 25 °C using a column bed volume of 3 ml and a sample size of up to 0.5 ml crude aqueous tissue extract (1:1 weight to volume). Equilibration and elution of unadsorbed proteins was conducted using a 0.05 M phosphate buffer, pH 7.0, containing 0.2 M NaCl. Elution of adsorbed material was performed using this same buffer containing 10% *N*-acetylglucosamine. These two fractions were concentrated and dialysed before electrophoresis. (The methodology used for concanavalin A-Sepharose affinity chromatography was similar, but in this case the buffers were 0.1 M acetate pH 6.0 containing 1 M NaCl, 1 mM MgCl₂, 1 mM MnCl₂, and the same buffer containing 20% methyl α -mannopyranoside for elution.)



appropriately linked *N*-acetylglucosamine residues and is converted into the *d*₂ isozyme, and the other, *a*₁, without the appropriate *N*-acetylglucosamine, which is converted into *d*₁. Both forms apparently contain sialic acid which is accessible to neuraminidase.

Further experiments using two-stage chromatographic separations involving both wheat germ lectin and concanavalin A indicate that there are several forms of the *d* isozyme in human tissues: *d*_{1a} with affinity for concanavalin A, but not wheat germ lectin; *d*_{1b} with affinity for neither lectin and *d*_{1c} and *d*₂ with affinity for both lectins. These isozymes occur in varying amounts in different tissues. It therefore seems likely that the so-called *a*, *b*, *c* and *d* isozymes each represent a multiplicity of forms with overlapping electrophoretic mobilities which differ only in their carbohydrate side chains.

The tissue ADA *e* isozyme does not fit readily into this scheme—our evidence suggests that it may not be a glycoprotein. It is also unusual in its restricted tissue distribution, occurring only in large amounts in the intestine, and its much larger apparent molecular size (435,000) (ref. 3) than the other tissue isozymes (230–280,000) (refs 3,5). It is possible that another protein converting factor is involved in the generation of the *e* isozyme from red cell ADA or the *e* isozyme may be coded by a separate structural locus. An alternative and perhaps simpler explanation is that the (glyco) protein which leads to the conversion of red cell ADA into the *a*, *b*, *c* and *d* isozymes is also responsible for the generation of the tissue ADA *e* isozyme, but the composition of the sugar chains may be different.

DALLAS M. SWALLOW
LORRAINE EVANS
D. A. HOPKINSON

MRC Human Biochemical Genetics Unit,
The Galton Laboratory, University College London,
Wolfson House, 4 Stephenson Way, London NW1, UK

Received 13 June; accepted 27 July 1977.

- ¹ Spencer, N., Hopkinson, D. A. & Harris, H. *Ann. hum. Genet.* **32**, 9 (1968).
- ² Hopkinson, D. A. & Harris, H. *Ann. hum. Genet.* **33**, 81 (1969).
- ³ Edwards, Y. H., Hopkinson, D. A. & Harris, H. *Ann. hum. Genet.* **35**, 207 (1971).
- ⁴ Giblett, E. R., Anderson, J. E., Cohen, F., Pollara, P. & Meuwissen, H. J. *Lancet* **ii**, 1067 (1972).
- ⁵ Hirschhorn, R., Levitsky, V., Pollara, B. & Meuwissen, H. J. *Nature new Biol.* **246**, 200 (1973).

- ⁶ Chen, S. H., Scott, C. R. & Giblett, E. R. *Am. J. hum. Genet.* **26**, 103 (1974).
- ⁷ Hirschhorn, R. *J. clin. Invest.* **55**, 661 (1975).
- ⁸ Akedo, H., Nishihara, H., Shinkai, K., Komatsu, K. & Ishikawa, S. *Biochim. biophys. Acta* **276**, 257 (1972).
- ⁹ Nishihara, H., Ishikawa, S., Shinkai, K. & Akedo, H. *Biochim. biophys. Acta* **302**, 429 (1973).
- ¹⁰ Lloyd, K. O., Kabat, E. A. & Beychok, S. *J. Immun.* **102**, 1354 (1969).
- ¹¹ Krusius, T., Finne, J. & Rauvala, H. *FEBS Lett.* **71**, 117 (1976).
- ¹² Greenaway, P. J. & LeVine, D. *Nature new Biol.* **241**, 191 (1973).

Translation of human immunoglobulin heavy chain mRNA *in vitro*

MOUSE immunoglobulin (Ig) mRNAs have been the subject of intensive study. Purification of Ig light chain mRNAs from myeloma or plasmacytoma cells has been monitored by translation *in vitro*, and studies have been directed towards sequence determination and gene titration. Moreover, partial purification of Ig heavy chain mRNA has also been achieved¹⁻³. No comparable work on the Ig mRNA of any other species has yet been published. We report here the preparation, fractionation and translation *in vitro* of mRNA from cells of a cloned normal human B lymphocytic cell line, RPMI 1788 (ref. 6), grown in tissue culture. Human Ig μ chains were identified in the translation product by specific immunoprecipitation and electrophoretic mobility in sodium dodecyl sulphate (SDS)-urea polyacrylamide gels.

Preliminary experiments were carried out on a range of human lymphocytic cell lines supplied by Searle Research Laboratories, High Wycombe, and RPMI 1788 was chosen for this work because it produced the highest level of Ig (5%–10% of total protein synthesis) in tissue culture. All of this Ig synthesis in RPMI 1788 is of the IgM type, that is, μ heavy chains and λ light chains. Analysis of the Ig production *in vivo* is shown in Fig. 1. The feasibility of using this cell line for the preparation of Ig mRNA was further indicated by the observation that the rate of protein synthesis and the level of IgM production are maintained when cells are grown in medium containing swine serum rather than the more costly foetal calf serum, and also when the cells are grown to a high density (up to 8×10^5 cells per ml) in suspension culture.

Total RNA was prepared from about 5 g wet weight

RPMI 1788 cells, freshly collected, essentially following the method of Bedard and Huang⁷. The total yield of 6 mg RNA was fractionated in sucrose gradients into a high molecular weight (HMW) fraction (5.5 mg) and a low molecular weight (LMW) fraction (0.5 mg) as shown in Fig. 2. The RNA in each of these pools was passed through

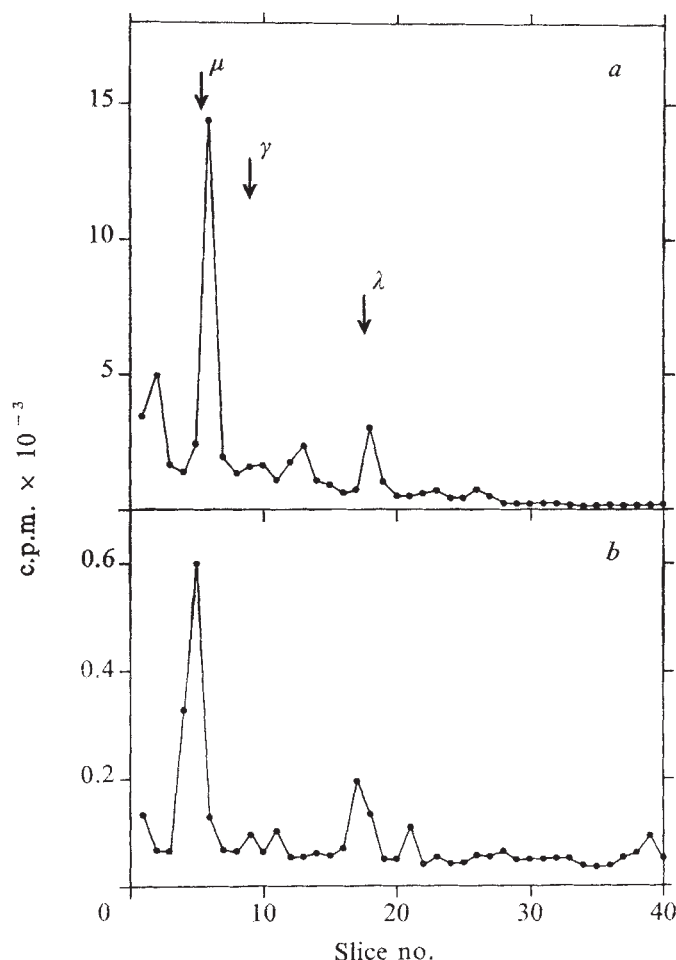


Fig. 1 Polyacrylamide gel electrophoresis identifying Ig chains synthesised by RPMI *in vivo*. RPMI 1788 cells (10^7) suspended in 2 ml RPMI 1640 medium containing 250 μ Ci ³H-leucine were incubated at 37 °C for 6 h, collected and lysed in 1 ml lysis buffer containing 0.5% Nonidet P40, according to the method of Prekumar *et al.*^{7,8}. Antibody precipitations were performed on 0.5 ml aliquots of the cell lysate and on 1 ml aliquots of the labelled medium as follows: 6 μ l rabbit antiserum to human μ -chain or to bovine serum albumin (Miles) were added and the samples incubated on ice 45 min before the addition of 15 μ l goat anti-rabbit IgG antiserum (Miles). Samples were incubated a further 45 min on ice and the immunoprecipitates were then collected, washed three times with phosphate buffered saline and once with ethanol-ether (1:1) containing 1% β -mercapto-ethanol, dried in a vacuum, and then dissolved and reduced by the addition of 50 μ l 6 M urea, 10 mM sodium phosphate, pH 7.5, 2% sodium dodecylsulphate (SDS), 1% β -mercapto-ethanol, 10% sucrose and incubation at 90 °C for 2 min. Each sample was electrophoresed in 10% polyacrylamide disc gels (6 mm $\times$ 6 cm) containing 6 M urea, 0.1 M sodium phosphate, pH 7.5, and 0.4% SDS, for 16 h at 2.5 mA per gel with a reservoir buffer of 0.1 M sodium phosphate, pH 7.5, 0.1% SDS and 5 mM thioglycolic acid. Gels were frozen and sliced at 1 mm intervals and each slice was incubated in 0.3 ml Soluene 350 (Packard) and the radioactivity monitored in toluene scintillant. Panels show anti μ -chain immunoprecipitates of the cell lysate (a), and of the medium (b). Unlabelled human μ , γ , and λ chains were run in a parallel gel and their positions at the end of electrophoresis are indicated. A similar gel of the anti-bovine serum albumin immunoprecipitate (not shown) revealed no μ or λ chains.

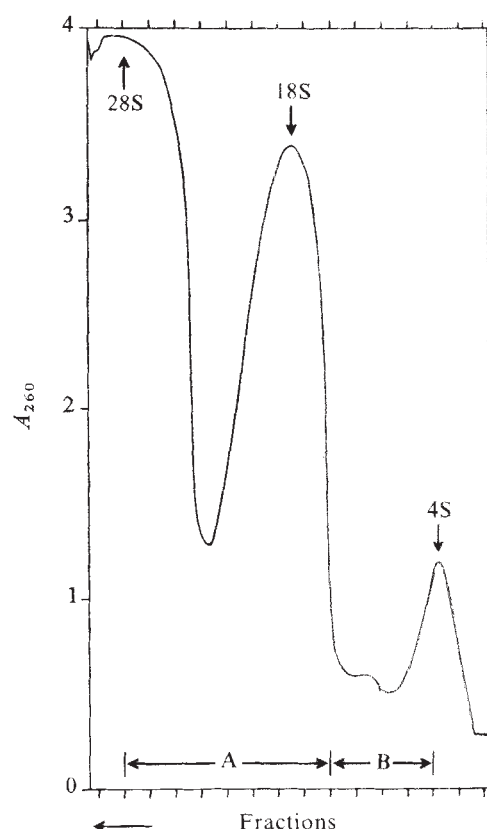


Fig. 2 Sucrose gradient fractionation of RPMI 1788 microsomal RNA. Microsomal RNA was prepared and sedimented through sucrose gradients using the method of Bedard and Huang⁹. All manipulations were at 4 °C unless otherwise indicated. The cells were homogenised by six strokes in a tight fitting glass-Teflon homogeniser in 5 volumes of 4 mM Tris (pH 7.4), 1.5 mM MgCl₂, 25 mM KCl, 250 mM sucrose. Nuclei and cell debris were pelleted at 3,000g for 10 min and the supernatant was removed and centrifuged at 30,000 r.p.m. (100,000g) for 20 min in a Beckman SW 41 rotor. The resultant microsomal pellet was dissolved in 2 volumes 5 mM Tris buffer, 3% SDS, at room temperature and immediately extracted twice on ice with 1 volume of phenol and subsequently 1 volume chloroform. The RNA was precipitated from the aqueous phase by addition of ethanol, washed, dissolved in water and layered on four 5%-30% sucrose gradients made in 0.1 M NaCl, 10 mM Tris (pH 7.5), 1 mM EDTA, 0.5% SDS. Gradients were centrifuged at 25,000 r.p.m. in a Beckman SW 41 rotor at 25 °C for 16 h, and then were fractionated by displacement from the bottom with 40% sucrose, while the fractionation was monitored with an Isco UA-4 (see trace). The fractions were pooled as indicated and the RNA in each pool was collected by ethanol precipitation, washed, and purified on oligo dT-cellulose (T-3; Collaborative Research) at room temperature in the presence of 10 mM Tris buffer and 0.5% SDS. The poly-A⁺ RNA, as well as the poly-A⁻ RNA, from each gradient were collected and washed as above, re-dissolved in water and stored at -20 °C.

a column of oligo dT-cellulose to separate the polyadenylated (poly-A⁺) RNA, presumably mRNA, from the non-polyadenylated (poly-A⁻) RNA. The HMW pool of Fig. 2 yielded 80 μ g poly-A⁺ and 2.1 mg poly-A⁻ RNA; the LMW pool yielded 18 μ g poly-A⁺ and 330 μ g poly-A⁻ RNA.

Three of these RNA fractions were analysed in 250 μ l rabbit reticulocyte lysates for their ability to stimulate protein synthesis *in vitro*. HMW poly-A⁺ RNA (25 μ g), LMW poly-A⁺ RNA (15 μ g), and HMW poly-A⁻ RNA (50 μ g), added to the assay system stimulated the incorporation of labelled amino acid to levels of 5,470, 3,031, and 708 c.p.m. above background (background=1,291 c.p.m.) per 2 μ l aliquot. Each of the incubated lysates was precipitated with antiserum to human μ chain. Only the HMW poly-A⁺ RNA stimulated lysate contained specifically immuno-

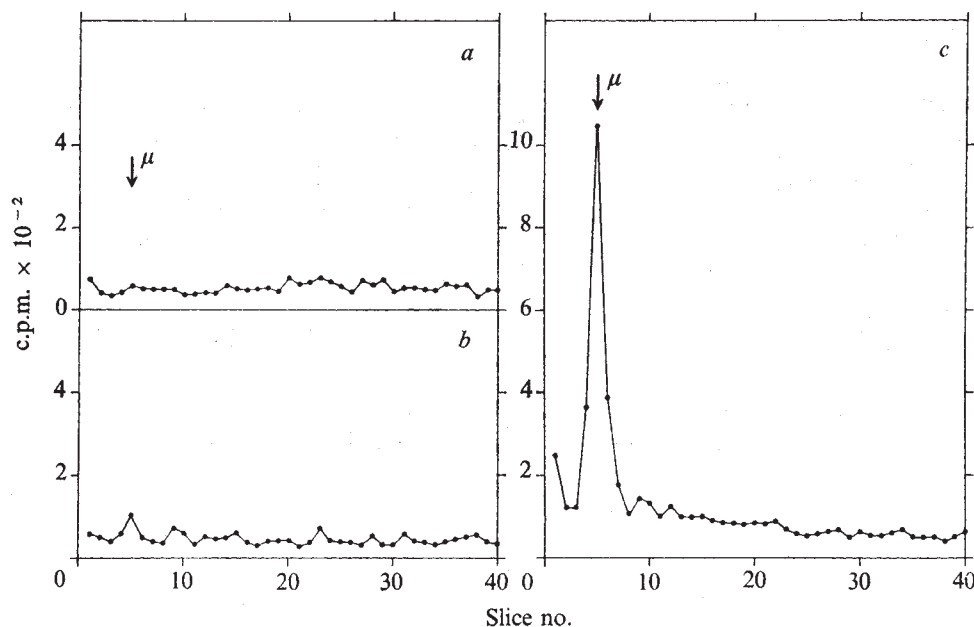


Fig. 3 Polyacrylamide gel electrophoresis demonstrating Ig μ -chain mRNA from human lymphocytes translated *in vitro*. RNA samples were analysed in 250 μ l rabbit reticulocyte lysates prepared by the method of Palmiter⁹, but buffered with 14 mM HEPES, pH 7.6, stored at -80°C , and pretreated with micrococcal nuclease by the methods of Pelham and Jackson¹⁰. After addition of 22 μCi ^{35}S -methionine and the RNA to be tested, each lysate was incubated for 90 min at 26°C . Aliquots of 2 μ l were precipitated with trichloroacetic acid and precipitates were washed, dried and monitored for radioactivity as a measure of total protein synthesis. To determine the quantity of μ chain synthesised, the remainder of each 250- μ l lysate was immunoprecipitated as follows: lysates were each mixed with 50 μ l 50 mM methionine, 25 μ l 10% Triton X-100, 25 μ l 10% sodium deoxycholate, and 4 μ l human IgM (8.7 mg ml^{-1}) or, as a control, 9 μ l ovalbumin (1 mg ml^{-1}) and centrifuged at 16,000g for 5 min. From each sample the top 225 μ l was removed and added to 50 μ l anti-human μ -chain antiserum (Miles) or 50 μ l anti-ovalbumin antiserum

(Miles). After incubation at 37°C , for 90 min each sample was layered over a discontinuous sucrose gradient (100 μ l 1 M sucrose + 50 μ l 0.5 M sucrose) in a 400 μ l Beckman microfuge tube and centrifuged at 16,000g for 5 min. The tubes were frozen at -80°C and the tip of each was cut off in order to collect the antibody precipitates, which were then dissolved, reduced, monitored for radioactivity and electrophoresed in 10% polyacrylamide disc gels as described in the legend to Fig. 1. The gel patterns shown represent the anti- μ -chain immunoprecipitates obtained from lysates containing 60 $\mu\text{g ml}^{-1}$ LMW poly-A⁺ RNA (a), 200 $\mu\text{g ml}^{-1}$ HMW poly-A⁺ RNA (b), and 100 $\mu\text{g ml}^{-1}$ HMW poly-A⁺ RNA (c). The gel pattern (not shown) of the anti-ovalbumin immunoprecipitate from a lysate containing 100 $\mu\text{g ml}^{-1}$ HMW poly-A⁺ RNA is identical to patterns (a) and (b). The gel pattern (not shown) of the anti-ovalbumin immunoprecipitate from a lysate containing 200 $\mu\text{g ml}^{-1}$ oviduct polysomal RNA shows one labelled peak well separated from the μ -chain position and co-migrating with the unlabelled ovalbumin marker.

precipitable protein. The amount of radioactivity recovered in the immunoprecipitate (multiplied by 3 since only one-third of the HMW poly-A⁺ RNA was tested in the assay) equals 0.7% of the total protein synthesised in response to the three RNA fractions (obtained as the sum of the correspondingly normalised activities). Gel electrophoresis of the immunoprecipitates in Fig. 3 shows that the protein precipitated by the μ -chain antiserum is homogeneous, and identical in mobility to human μ -chain marker. These results indicate the presence of intact, translatable μ -chain mRNA in the HMW poly-A⁺ fraction of RNA prepared from human lymphocytes.

The cells used in the experiments described above were freshly collected, but this does not seem to be essential. The entire procedure has been repeated using a similar quantity of cells which had been rapidly frozen in liquid N_2 as a cell pellet and stored at -80°C before RNA preparation. The results of the second experiment, including yields of RNA, the sedimentation pattern of the RNA, the yields of poly-A⁺ and poly-A⁻ RNA and the ability of the HMW poly-A⁺ fraction to stimulate the *in vitro* synthesis of anti- μ chain precipitable protein, were essentially identical to the results with freshly collected cells illustrated in Figs 2 and 3. The second preparation yielded a percentage of anti- μ -chain precipitable protein of 3.4%, which approaches the level of 5%–10% estimated for *in vivo* synthesis.

In this second experiment, the 18S peak from the sucrose gradient was pooled separately from the rest of the HMW fraction. Immunoprecipitation of the *in vitro* translation products showed that more than 90% of the μ -chain mRNA is located in the 18S fraction, indicating that this mRNA has a sedimentation coefficient of around 18S. This result may be compared with reports that, in mouse, the α -heavy chain mRNA (from MOPC 315 plasmacytoma) sediments at

16–19S (ref. 3), and γ -chain mRNA (from MOPC 21 tissue culture cells) at 17S (ref. 1), and suggests that the human μ -heavy chain mRNA may be of similar size. Work is now in progress to prepare large quantities of RPMI 1788 RNA to be used for the further physical and chemical characterisation of the Ig mRNA.

We thank Dr Brian Richards, Dr John Birch and Mr David Edwards of Searle Research Laboratories for support and collaboration, Dr Ru Chih Huang for advice, and Mr Paul Singer for help with preliminary experiments. This work was supported by MRC grant G969/509/B, USNIH postdoctoral fellowship 1-F32 GM 05558-01 (C.K.K.), and a King's College tutorial fellowship (F.C.).

CAROL K. KLUKAS

FRANS CRAMER

HANNAH GOULD

Department of Biophysics,
King's College,
26–29 Drury Lane,
London WC2, UK

Received 31 May; accepted 27 July 1977.

- Cowan, N. J. & Milstein, C. *Eur. J. Biochem.* 36, 1–7 (1973).
- Cowan, N. J., Secher, D. S. & Milstein, C. *Eur. J. Biochem.* 61, 355–368 (1976).
- Green, M., Graves, P. N., Zehavi-Willner, T., McInnes, J. & Pestka, S. *Proc. natn. Acad. Sci. U.S.A.* 72, 224–228 (1975).
- Green, M., Zehavi-Willner, T., Graves, P. N., McInnes, J. & Pestka, S. *Archs Biochem. Biophys.* 172, 74–89 (1976).
- Bedard, D. L. & Huang, R. C. *J. biol. Chem.* 252, 2592–2598 (1977).
- Moore, G. E., Gerner, R. E. & Franklin, H. A. *J. Am. med. Ass.* 199, 519 (1967).
- Premkumar, E., Potter, M., Singer, P. A. & Sklar, M. D. *Cell* 6, 149–159 (1975).
- Premkumar, E., Singer, P. A. & Williamson, A. R. *Cell* 5, 87–92 (1975).
- Palmiter, R. D. *J. biol. Chem.* 248, 2095–2106 (1973).
- Pelham, H. R. B. & Jackson, R. J. *Eur. J. Biochem.* 67, 247–256 (1976).

Identification of chromosomal location of yeast DNA from hybrid plasmid *pYeleu10*

THERE is strong evidence to suggest that some genes of baker's yeast *Saccharomyces cerevisiae* can be expressed in *Escherichia coli* when cloned on plasmid or phage vectors^{1,2}. This conclusion is based on the observation that hybrid plasmids and bacteriophage carrying specific fragments of yeast nuclear DNA can complement certain auxotrophic mutations in the *E. coli* genome. Complementation alone, however, is not sufficient to prove that the cloned DNA contains a yeast gene equivalent to the defective bacterial gene. One aspect of the identification of any cloned DNA is the identification of its chromosomal origin. We present here a method for the identification of the chromosomal origin of cloned yeast DNA using hybridisation to DNA from yeast strains aneuploid for a single chromosome. Using this method we have confirmed the chromosomal location of the DNA of a yeast hybrid plasmid (*pYeleu10*) presumed to contain sequences coding for the leucine biosynthetic enzyme β -isopropylmalate dehydrogenase, the *leu2* gene of yeast.

Ratzkin and Carbon¹ attempted to isolate a hybrid plasmid containing a functional yeast *leu2* gene by transformation of a *leu*⁻ bacterial strain (JA199; *leuB6*, *trpE5*, *lacY*⁻ *hsdR*⁻ *hsdM*⁻ *F*⁺) defective in β -isopropylmalate dehydrogenase to *leu*⁺ using randomly sheared yeast DNA cloned on the plasmid vector *ColE1*. These workers isolated several independent clones which contained plasmids capable of suppressing the *leuB*⁻ defect in the host cell. Analysis of the restriction patterns of the plasmid DNA from these clones gave the surprising result that at least four distinct yeast DNA sequences were represented among these plasmids. As only one locus (*leu2*) is known in yeast to code for the enzyme β -isopropylmalate dehydrogenase, this result meant that in at least some cases suppression of the host

leuB mutation could occur by means other than enzymatic complementation. This result emphasises that complementation does not provide clear evidence for the presence of a specific functional gene or a unique segment of DNA corresponding to the yeast *leu2* region. Further genetic tests by these workers showed that one plasmid, *pYeleu10*, complemented several *leuB*⁻ alleles while the rest were specific for the original allele, *leuB6*. For this reason it seemed likely that plasmid *pYeleu10* might be expressing the cognate yeast gene, *leu2*, which is located on the left arm of chromosome III, near the centromere.

To determine unambiguously the chromosomal location of the yeast DNA carried by *pYeleu10*, we have analysed the hybridisation of labelled plasmid DNA to yeast DNA from strains aneuploid for chromosome III. Hybridisation was carried out after transferring restriction digests of the yeast DNA to nitrocellulose filters by the Southern blotting procedure³. The rationale for the experiment was that in the conditions used (that is, excess probe) the amount of label bound should be directly related to the amount of DNA originally present on the filter, and therefore that the degree of hybridisation to bands from chromosome III should reflect the aneuploid condition. The yeast strains used are described in Table 1 and include a normal haploid, a normal diploid, a diploid monosomic for chromosome III (2*n*-1) and a haploid disomic for chromosome III (*n*+1). Thus, the amount of chromosome III DNA per haploid genome (*n*) in each strain varied from *n*/2 (H151-2A) to *n* (+D4, 8481) to 2*n* (H159/1). It should be emphasised that the success of this and similar experiments depends on an accurate determination of the genotype of the aneuploid strains at the time of DNA isolation. Aneuploidy in most organisms, including yeast, is an unstable condition and strains may frequently revert to euploidy during culture. Genetic assays for aneuploidy in strains H159/1 and H151-2A are described in the legend to Table 1. Methods used for isolation and labelling of DNA along with conditions for hybridisation are described in the legend to Fig. 1. The hybridisation probe consisted of a mixture of ³²P-labelled

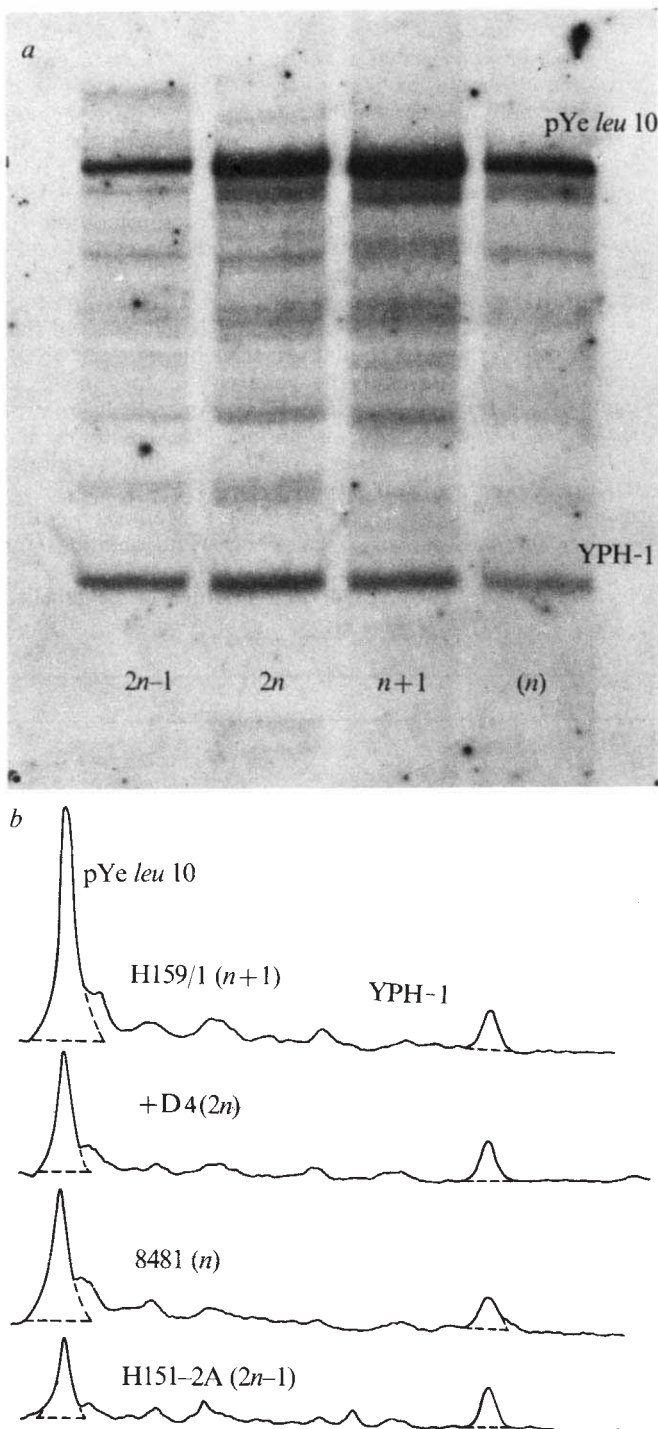
Table 1 Yeast strains used

Strain	Chromosome no.	Genotype	Copies of chromosome III per haploid genome
A364a +D4	2 <i>n</i>	<i>ura1 ade1 ade2 a</i> <i>ura1 ade1 ade2 a</i>	1
8481	<i>n</i>	<i>his4-260 a HOL1-1</i>	1
H159/1	<i>n</i> +1	<i>his4-38 leu2-1_{oc} α +</i> <i>his4-280 leu2-1_{oc} a SUP61</i>	2
H151-2A	2 <i>n</i> -1	<i>his4- leu2-1 α thr4 mal2</i> <i>lys1-1 ade2-1</i> + +	½
S36	2 <i>n</i> -1	<i>a ino1-13 ino4-8</i> <i>ino1-13 ino4-8</i>	½

Strains were constructed in this laboratory by standard genetic techniques⁶ with the exception of A364a +D5 which was obtained from Lee Hartwell. The media used were described previously⁷. Cultures of H159/1 and H151-2A used for preparation of yeast DNA were tested for aneuploidy immediately before collecting as follows. H151/1: the culture was diluted and plated on YEPD (complete) agar for single colonies and tested for expression of genetic markers by replica plating. Because of the presence of the ochre suppressors *SUP61* (Hawthorne, personal communication) the phenotype of a colony arising from an aneuploid cell of the genotype shown in this Table will be *Leu*⁺ *Lys*⁺ *His*⁻ *Met*⁻ *canavanine* sensitive (*Can*^s) and non-mating (*a/a*). But, because of the spontaneous loss of the chromosome containing the suppressor, some cells in the colony exhibit an altered phenotype, *Leu*⁻ *Lys*⁻ *His*⁻ *Met*⁻ *Can*^r and mating type *α*. Such segregants can be detected by plating on medium containing canavanine and subsequently testing the *Can*^r segregants for the other nutritional markers and mating type. The presence of these segregants in a clone provides evidence that the parent cell was aneuploid. All 289 colonies tested exhibited all aspects of this complex phenotype and the culture was assumed to contain virtually 100% aneuploid (*n*+1) cells. H151-2A: an aliquot of this culture was mated with cells of strain S36 on YEPD agar, then transferred to SPOR agar to induce meiosis and sporulation. Tetrads were dissected and assayed for each of the genetic markers in the cross. Because the two strains were mated *en masse*, resulting in the formation of thousands of independent zygotes, it was assumed that each tetrad was the result of an independent mating event and thus reflected the genotype of an individual cell from the H151-2A culture. Tetrads resulting from matings involving aneuploid H151-2A cells would be expected to exhibit 2:2 segregation for each of the alleles located on chromosome III. But, tetrads exhibiting 4:0 or 3:1 segregation for mating type, cryptopleurine resistance (*Cry*^r) or maltose fermentation would indicate that two copies of each of these alleles were present and that parent H151-2A cell had reverted to euploidy. Of 83 tetrads, 70 scored were of the former type (2:2) indicating that the culture of H151-2A from which DNA was isolated contained at least 80% aneuploid (2*n*-1) cells. *Lys1-1* and *ade2-1* exhibited normal tetraploid segregation.

DNA from *pYeleu10* and *YPH-1*, a hybrid plasmid created in this laboratory containing an unidentified segment of yeast DNA. *YPH-1* was used as an internal standard for the extent of hybridisation. Although its chromosomal location is not known, it is presumed not to be located on chromosome III because hybridisation of *YPH-1* to whole yeast DNA is not affected by aneuploidy of chromosome III (J.H., unpublished). *Hind* III was used to digest the yeast DNA because neither *pYeleu10* nor *YPH-1* are cut by this enzyme and each should thus be represented by a single band in a yeast *Hind* III digest.

The results of one hybridisation experiment are shown in Fig. 1. The degree of hybridisation in each of the two main bands was assayed by cutting out and weighing the two main peaks in the densitometric tracings. As shown in Table 2, the ratio of the peak areas in each track varies



directly with the ratios of chromosome III per haploid genome. This result was confirmed by cutting the bands out of the filter and assaying radioactivity in a scintillation counter (not shown). From these data we conclude that *pYeleu10* carries sequences located on chromosome III. This experiment has been repeated with similar results (not shown).

An intriguing feature of the radioautograph obtained by this procedure is that faint bands are observed in each track in addition to the single main band associated with each plasmid. These are present even when *pYeleu10* alone is used as probe and have been frequently observed by other workers (K. Struhl and J. Carbon, personal communication). It is not likely that they are the result of incomplete digestion of the yeast DNA since some of them are smaller than the smallest *Hind* III fragment which could correspond to *pYeleu10*, that is, a fragment equal in size to *pYeleu10* itself since *pYeleu10* contains no *Hind* III restriction sites. It is tempting to speculate that these 'ghost bands' might represent partially homologous sequences scattered throughout the genome which have a common regulatory or structural function.

We conclude that plasmid *pYeleu10* contains sequences from chromosome III of yeast, probably including the *leu2* locus. Using this plasmid as a probe it will be possible to locate sequences flanking the *leu2* region among collections of clones containing yeast DNA and by repeating that process to 'walk' down the chromosome to other regions of

Fig. 1 a, Radioautograph of ^{32}P -labelled DNA from plasmids *pYeleu10* and *YPH-1* hybridised to a 'Southern blot' (ref. 3) containing *Hind* III restriction digests of yeast DNA. **b**, Densitometer tracings of the individual tracks in the radioautograph. Yeast DNA was prepared by the method of Cryer, Eccleshall and Marmur⁸. Approximately 2 μg of DNA from each strain was digested with restriction endonuclease *Hind* III (New England Biolabs; 2 units per μg DNA, 37 °C for 4 h) and subjected to electrophoresis on a horizontal 0.8% agarose slab gel (1.3 $\times$ 15 cm tracks) for 12 h at 4 mA per track. The restriction digest in the gel was then transferred to a nitrocellulose filter (Millipore HAWP) by the Southern blotting procedure³. DNA from plasmids *pYeleu10* and *YPH-1* was isolated by a modification of procedures described by Clewell⁹ and Guerry *et al.*¹⁰. Cultures were grown in supplemented synthetic medium to mid-log phase ($2\text{--}4 \times 10^8$ cells per ml) at which time chloramphenicol was added to a concentration of 250 mg l^{-1} and the cultures were shaken at 37 °C for 25 h to allow amplification of the plasmid⁹. The culture was divided into 200-ml aliquots for further processing. Cells were collected by centrifugation, washed in 10 mM Tris-1 mM EDTA, pH 8.0 and resuspended in 2 ml 50 mM Tris-25% sucrose, pH 8.0 plus 2 mg lysozyme (Worthington). The mixture was incubated 5 min at 0 °C before addition of 0.4 ml EDTA, pH 8.0 and further incubation for 10 min at 0 °C. Lysing buffer (1% Triton X-100, 1 mM EDTA, 50 mM Tris, pH 8.0) 4 ml was added followed by incubation for an additional 15 min at 0 °C. The lysate was then centrifuged for 90 min at 18,000 r.p.m. in a Sorvall SS-34 rotor to pellet cell debris and most of the chromosomal DNA. The supernatant was then carefully decanted away from the loose pellet, volume was adjusted to 8.0 ml with 0.5 M EDTA and 0.3 ml ethidium bromide (10 mg ml^{-1}) was added. Protein precipitated by the CsCl-EtBr was removed by centrifugation at 30,000g and the cleared supernatant centrifuged for 40 h at 40,000 r.p.m. in a Beckman 65 rotor. The plasmid DNA band was extracted from the density gradient as described by Clewell⁹. Ethidium bromide was removed by three extractions with isopentyl alcohol followed by dialysis against 10 mM Tris-1 mM EDTA pH 7.5. Plasmid DNA was labelled with ^{32}P by nick translation using DNA polymerase I (Boehringer-Mannheim Grade I) according to the method of Maniatis *et al.*¹¹. Specific activity of the labelled plasmid DNA was 1.1×10^6 Ci μg^{-1} . After denaturation of the probe DNA in 0.2 M NaOH at 65 °C, hybridisation to the filter-bound DNA was accomplished in 10 ml of $4 \times \text{SSC} + 0.1\%$ sodium dodecylsulphate at 65 °C for 44 h. The filter was then exhaustively washed in 3 mM Tris base (M. Botchan, personal communication) and exposed to Kodak No-Screen X-ray film (NS2T) for 3h. The exposed film was then scanned using a densitometer. The sensitivity of the densitometer was adjusted for each scan to normalise approximately *YPH-1* peaks.

Table 2 Comparison of the peak areas in the densitometric tracings in Fig. 1

Strain	Weight of cut-out peak (mg) <i>pYeleu10</i>	Weight of cut-out peak (mg) <i>YPH-1</i>	Ratio: <i>pYeleu10</i> / <i>YPH-1</i>	Copies of chromosome III per haploid genome
H151-2A	17.1	10.2	1.4	$\frac{1}{2}$
8481	36.7	11.7	3.1	1
+D4	32.7	10.2	3.3	1
H159/1	66.8	11.6	5.8	2

Areas were measured by reproducing the densitometer tracings onto heavy paper, cutting out two copies of each peak (as indicated by the dotted lines) and weighing each one. Weights shown are the average of two measurements.

interest. It should further be possible to identify cloned DNA from specific genetic loci by hybridisation to restriction digests of DNA from yeast strains carrying genetically defined deletions which enter these loci. Southern blots of DNA from such strains should exhibit altered hybridisation patterns relative to wildtype DNA. Deletions exist which have endpoints in or near the *his4* locus⁴, the centromere (H. Klein and G. F., unpublished), and the mating type locus⁵. Attempts to isolate the DNA from these loci by this approach are under way.

We thank Barry Ratzkin and John Carbon for allowing us to use the *pYeleu10* plasmid and Dick Hallberg for informative discussion. This work was supported by NIH grant GM15408 (G.R.F.) and an NIH Post-doctoral Service Award (J.B.H.). All work involving living cells containing recombinant DNA was performed in accordance with the NIH Guidelines for Recombinant DNA Research¹².

JAMES HICKS
GERALD R. FINK

Section of Genetics, Development and Physiology,
Cornell University,
Ithaca, New York 14853

Received 31 May; accepted 18 July 1977.

- Ratzkin, B. & Carbon, J. *Proc. natn. Acad. Sci. U.S.A.* **74**, 487-491 (1977).
- Struhl, K., Cameron, J. R. & Davis, R. W. *Proc. natn. Acad. Sci. U.S.A.* **73**, 1471-1475 (1976).
- Southern, E. J. *molec. Biol.* **98**, 503-518 (1975).
- Fink, G. R. & Styles, C. A. *Genetics* **77**, 231-244 (1974).
- Hawthorne, D. C. *Genetics* **48**, 1727-1729 (1963).
- Mortimer, R. K. & Hawthorne, R. C. in *The Yeasts* (eds Rose, A. H. & Harrison, J. S.) 385-460 (Academic, New York, 1969).
- Hicks, J. & Herskowitz, I. *Genetics* **83**, 245-258 (1976).
- Cryer, D. R., Eccleshall, R. & Marmur, J. *Meth. Cell Biol.* **12**, 39-44 (1975).
- Clewell, D. B. J. *Bact.* **110**, 667-676 (1972).
- Guerry, P., LeBlanc, D. J. & Falkow, S. J. *Bact.* **116**, 1064-1066 (1973).
- Maniatis, T., Jeffrey, A. & Kleid, D. G. *Proc. natn. Acad. Sci. U.S.A.* **72**, 1185-1188 (1975).
- Fed. Regist.*, **41**, (no. 131) (1976).

Deuterium NMR study of lipid organisation in *Acholeplasma laidlawii* membranes

THE most convincing studies of molecular order in model membranes are undoubtedly those involving deuterium nuclear magnetic resonance (NMR) of labelled lipids in the lamellar liquid crystalline phase¹⁻⁶. The relevance of these results to biological membranes has not yet been established, however; there is no *a priori* reason to expect the state of organisation of lipids in the lamellar liquid crystal to resemble closely that of a natural membrane. We report here the first detailed study of acyl chain order in the lipids of a natural biological membrane—the plasma membrane of the microorganism *Acholeplasma laidlawii*. Specifically deuterated fatty acids were incorporated biosynthetically into the membrane lipids as described previously⁷. The ²H-NMR spectra of the membrane demonstrate the co-existence of at least two different kinds of lipid. Signals

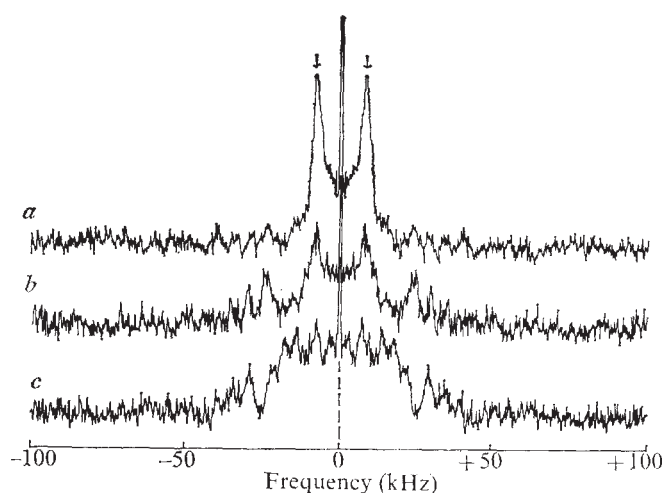
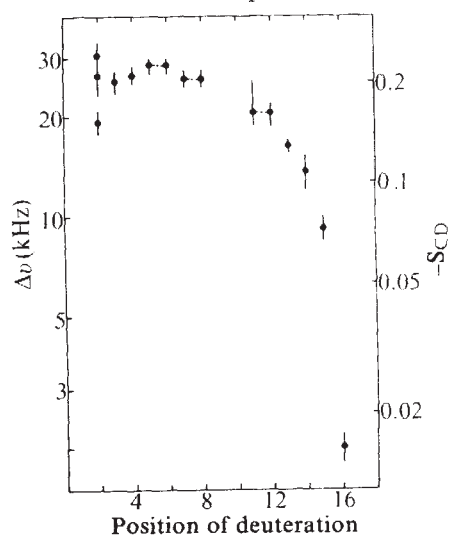


Fig. 1 ²H-NMR spectra, at 13.8 MHz, of *Acholeplasma laidlawii* membranes containing palmitate-13d₂. At 42 °C (a) the spectrum is dominated by the fluid phase with the splitting indicated by the two arrows. As the temperature is lowered (b, 30 °C; c, 25 °C), the spectrum appears more characteristic of a gel. All the spectra were recorded at a repetition rate of 4 scans per s. The number of scans was 120,000 at 42 °C and 40,000 at 30 °C and 25 °C. The spectra were symmetrised about the Larmor frequency which is represented by zero.

from the more fluid lipid are most prominent at temperatures near and above the growth temperature (37 °C). The more solid-like lipid, which predominates at lower temperatures, may be similar to the gel state of lamellar phases and could include lipid associated with the membrane protein. For the more fluid phase, the plot of order parameter against position of deuteration strongly resembles that for egg yolk² and dipalmitoyl³ phosphatidylcholine. Incorporation of cholesterol into the membrane of *A. laidlawii* increases the average acyl chain order and also increases the spread of order parameters, similar to its effect on egg yolk phosphatidylcholine⁶. These results represent a major justification for the use of lamellar liquid crystals as models for the lipid in biological membranes.

The NMR samples consisted of 0.5–1.3 g of lyophilised *A. laidlawii* membranes which were hydrated with 1–3 ml of distilled water. The average acyl chain composition of the membrane lipids was as follows: 12:0 = 6.6% (1.2); 14:0 =

Fig. 2 The distance between the peaks in the powder pattern, $\Delta\nu$, against position of deuteration of the palmitate chains at 42 °C. Points connected by dotted lines were obtained from samples labelled in two adjacent positions for which the two powder patterns are not resolved. Error bars are estimated from the spectra and do not include possible effects due to variation in membrane composition.



18.1% (2.3); 16:0 = 68.8% (3.6); minor components = about 6.5% as determined by gas-liquid chromatography. The values given represent mean compositions for all 10 specifically labelled samples. The numbers in parentheses are average deviations from the mean. Thus the membrane lipid chains were enriched to about 70% with specifically deuterated palmitate. The small random variation in chain composition, which seems to be unavoidable in this experiment, is independent of the position of deuteration in the palmitate chain.

The ^2H -NMR spectra of labelled biological membranes are characterised by quadrupolar 'powder' spectra spanning a wide frequency range and with residual quadrupolar splittings of 10^3 – 10^5 Hz (refs 2, 7). A quadrupolar spin echo technique ('solid echo') was used here to obtain undistorted spectra. The merits of this simple pulse technique, necessary to these experiments, have been discussed in detail by Davis *et al.*⁸. The signals were recorded at the ^2H Larmor frequency so that the low field half of the symmetrical quadrupolar powder spectrum appears folded and superimposed on the high field half, thus increasing the detection sensitivity by a factor of $\sqrt{2}$. To facilitate visual interpretation of these spectra, we plotted sample spectra reflected about the symmetry axis to simulate the appearance of a complete spectrum and show the quadrupolar splitting (Fig. 1). The reader should not be misled by the apparent perfect symmetry of these spectra.

As shown in Fig. 1, above the growth temperature of 37 °C, the ^2H -NMR spectra are dominated by a quadrupolar powder spectrum which resembles strongly those observed for simple liquid crystalline phospholipids^{2,3}. At lower temperatures, signals characteristic of a more solid-like phase appear. This behaviour is entirely consistent with the extremely broad thermal phase transition reported previously for *A. laidlawii* membranes derived from palmitate-supplemented media⁹. The quadrupolar splittings and related order parameters S_{CD} were measured as a function of the position of the deuterium label in the palmitate chain for the fluid phase of *A. laidlawii* membranes at 42 °C. A plot of the deduced order parameters S_{CD} against position of deuteration is shown in Fig. 2. This plot has the same characteristic shape as those found for lamellar liquid crystalline phases of single phospholipids^{2,3}. The 'plateau' region of constant order extends from carbon

2 to at least carbon 8 as for the lamellar phases, and the actual values for S_{CD} are comparable with those found for egg yolk and dipalmitoyl phosphatidylcholine^{2,3}. The important consequence of these findings is that inferences concerning acyl chain conformation and bilayer geometry, and their relationship to biological function, made previously for the lamellar phases^{2,3} apply also to *A. laidlawii* membranes.

The spectrum for membranes containing 2,2-dideuterio-palmitate is considerably different from that for any other label position. The unusual shape of the line suggests the presence of possibly three overlapping powder doublets. The quadrupolar splitting for this position is expected to be very sensitive to differences in the initial conformation of the two acyl chains¹⁰, and also to differences in the head groups (*A. laidlawii* lipids contain several different head groups).

The effect of incorporation of cholesterol into *A. laidlawii* membranes was investigated using fully deuterated palmitate chains. While this technique was highly successful in probing the effect of cholesterol on egg yolk phosphatidylcholine⁶, much less resolution of the NMR signals for individual chain positions was obtained for the membranes (see Fig. 3). Nevertheless, the general shape of the envelope due to numerous overlapping powder doublets can provide useful information⁸. The sharp edges of the envelope are characteristic of a system having a plateau region in the ordering profile¹¹ (Fig. 2). The effect of cholesterol is to spread the intensity of the NMR spectrum over a wider frequency range. This is consistent with the much greater ordering effect of cholesterol on the plateau region compared with the methyl end of the chain, as found previously for egg yolk phosphatidylcholine⁶. The increase in the quadrupole splitting from 30 to 50 kHz for the plateau region with 2:1 lipid:cholesterol ratio is very similar to that found for egg phosphatidylcholine⁶.

With the increased sensitivity of contemporary NMR techniques and the greater availability of deuterium-labelled lipids, ^2H -NMR provides an excellent method to probe the fine detail of molecular organisation in a biological membrane with a minimum of perturbation or approximation.

The research at UBC was supported by the National Research Council of Canada and by a special Killam-Canada Council Interdisciplinary Grant. J. H. D. is the holder of a NRC Postdoctoral Fellowship.

GERALD W. STOCKTON
KENNETH G. JOHNSON
KEITH W. BUTLER
A. P. TULLOCH
YVAN BOULANGER
IAN C. P. SMITH

Division of Biological Sciences,
National Research Council of Canada,
Ottawa, Ontario, Canada

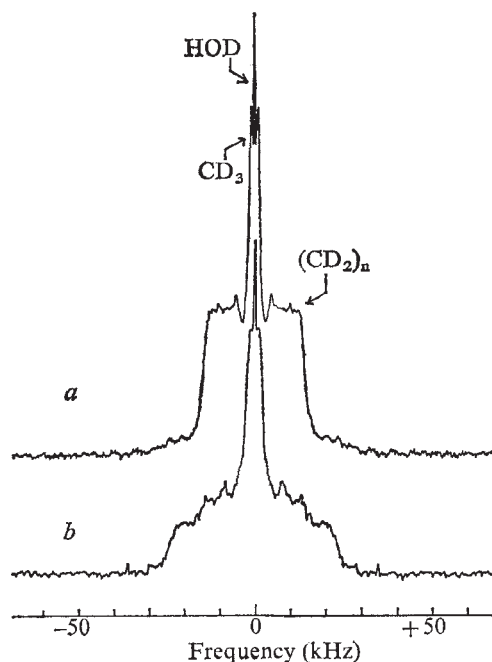
JAMES H. DAVIS
MYER BLOOM

Department of Physics,
University of British Columbia,
Vancouver, British Columbia, Canada

Received 21 April; accepted 11 July 1977.

- 1 Oldfield, E., Chapman, D. & Derbyshire, W. *FEBS Lett.* 16, 102–104 (1971).
- 2 Stockton, G. W., Polnaszek, C. F., Tulloch, A. P., Hasan, F. & Smith, I. C. P. *Biochemistry* 15, 954–966 (1976).
- 3 Seelig, A. & Seelig, J. *Biochemistry* 13, 4839–4846 (1974).
- 4 Mely, B., Charvolin, J. & Keller, P. *Chem. Phys. Lipids* 15, 161–173 (1975).
- 5 Marcelja, S. *J. chem. Phys.* 60, 3599–3604 (1974).
- 6 Stockton, G. W. & Smith, I. C. P. *Chem. Phys. Lipids* 17, 251–263 (1976).
- 7 Stockton, G. W. *et al. Biochim. biophys. Acta* 401, 535–539 (1975).
- 8 Davis, J. H., Jeffrey, K. R., Bloom, M. & Valic, M. I. *Chem. Phys. Lett.* 42, 390–394 (1976).
- 9 Steim, J. M., Tourtellotte, M. E., Reinert, J. C., McElhaney, R. N. & Rader, R. L. *Proc. natn. Acad. Sci. U.S.A.* 63, 104–109 (1969).
- 10 Seelig, A. & Seelig, J. *Biochim. biophys. Acta* 406, 1–5 (1975).
- 11 Davis, J. H. & Jeffrey, K. R. *Chem. Phys. Lipids* (in the press).

Fig. 3 ^2H -NMR spectra, at 13.8 MHz and 42 °C, of *A. laidlawii* membranes containing perdeuteriopalmate; *a*, without cholesterol and *b*, with 2 : 1 lipid : cholesterol ratio. As in Fig. 1, the spectra were made symmetrical about the Larmor frequency.



reviews

Energy crisis

John Chesshire

Energy: The Continuing Crisis. By Norman Metzger. Pp. 242. (Thomas Y. Crowell: New York, 1977.) \$12.95.

As if to anticipate the reviewer, the author commences his study by questioning the need for yet another book on the "energy crisis". The justification lies in his belief that since 1973, energy consumers have been lulled into a dangerous complacency: new finds of fossil fuels are reported daily, and the nuclear and non-conventional technology lobbies sing in high praise of the potential of their systems. This appearance of plenty allows consumers the luxury of using yet more fuel while putting up stern and vocal criticism to planning applications for new nuclear stations, oil refineries and coal mines. Meanwhile, their elected representatives, whether in the US Congress or in the British House of Commons, point to the political implications of tough conservation measures, especially any which might bring about the transition of fuel prices to the long-term marginal costs of replacements. Thus, the "continuing crisis", and the need for another book.

The author adopts the now-common, fuel-by-fuel approach in his analysis, and focuses on the US situation. He points to the steady demise of coal this century, and to the massive injection of funds now required to raise production by providing new capacity and efficient mining, conversion and utilisation technologies. He concludes by arguing that the scale of the resource requirement is such that the vast expansion of the coal industry is by no means the certainty it seems to be in current energy policy planning.

Critical to his review of the oil sector is an analysis of the likelihood of continued cohesion of the OPEC cartel (a fixation with many American commentators). The absence of any discussion of the role of the state in determining alternative depletion policies is a weakness here, as in the succeeding chapter on natural gas. His useful arm's-length account of the "Troubled Youth of Nuclear Power" includes an assessment of recent American action and reaction in the build-up of nuclear generating capacity, and a damning indictment of the lack of attention to waste disposal

and storage, and leads him to the whimsical (but incisive) view that "maybe the trouble with nuclear reactors is that they don't have belching smokestacks".

Of the unconventional technologies, he anticipates that the major contribution will come from the fusion, solar and geothermal routes, and he highlights the critical uncertainties regarding the cost, scale and timing of their contribution to future energy supplies.

On balance, the author is to be commended for his realistic evaluation of the many exaggerated claims that have

been made about both fossil and novel energy supplies, and for his sensitive analysis of the constraints on change imposed by the power and vested interests of the energy corporations and the regulatory functions of the state. It is unfortunate that he favours a supply—rather than a demand—orientated approach, thus neglecting conservation policies and technologies and that he adopts a relatively short-term US-centred view of the world. □

John Chesshire is a Fellow of the Science Policy Research Unit, University of Sussex, UK.

Tracing the history of life

The Phylogeny of the Vertebrata. By Søren Løvtrup. Pp. xii+330. (Wiley: London and New York, 1977.) £15; \$29.50.

COMPARATIVE BIOLOGY has been revitalised in recent years under the stimulating influence of Willi Hennig's *Phylogenetic Systematics*. No longer can it be regarded as the feeble survivor of a primitive descriptive phase of biology, for it is evidently central to evolutionary biology and gives meaning and direction to research in fields as diverse as embryology, population genetics, biogeography and behaviour. It is based on a belief in the objective existence of a true path of descent for all living organisms which may be reconstructed by the empirical testing of well formed hypotheses. Nevertheless, despite its already long life, comparative biology is not yet a mature science with well understood philosophical and theoretical foundations. Søren Løvtrup's book attempts to remedy this defect, using the *Vertebrata* to exemplify his ideas.

The book opens by setting out its Popperian research programme and then immediately takes up the "Logic of Phylogenetics". By excluding fossils and thereby avoiding argument about what is in any case a practical rather than a theoretical difficulty, Løvtrup is able to treat classification and phylogeny reconstruction as the same process. Thus, of the two levels of taxonomic activity differentiated by Gregg—taxonomy proper and method-

ological taxonomy—Løvtrup attacks the latter. Taxonomy at the higher level is a primitive science. As Gregg says, "Taxonomists engaged in methodological research are [thus] without adequate linguistic resources to support their investigations. They must rely almost entirely upon unsuitable prescientific idioms borrowed from everyday language". Gregg's own solution was the use of set theory, but this had no hope of acceptance in a pre-Hennigian intellectual climate that deliberately depressed rigour and allowed artifice and authority to flourish. Twenty-five years later, Løvtrup is in a more favourable position, though it is perhaps unfortunate that his "language" is a series of definitions, axioms and theories, rather than the symbolic logic of Gregg.

The heart of Løvtrup's book comprises two long chapters on the ancestry and divergence of vertebrates. These are more easily approached than other chapters and will no doubt readily attract attention because of their heterodox conclusions. However, Løvtrup has more important things to say elsewhere in this book, and as he deviates so disastrously from his professed deductive approach, I am disinclined to give much weight to his conclusions. The last section of the book deals with the mechanism of evolution. Comparative biology has not benefitted greatly at a practical level from work in this field, and Løvtrup tries to redress the balance with an axiomatised version of his "comprehensive" theory. This is

distinguished by the importance it attaches to large step changes and the pressure of macromutations, over and above natural selection.

How well has Løvtrup succeeded in clarifying these important and difficult issues? Reluctantly, I must suggest that in large measure he has been unsuccessful, though whether this is due to the unsuitability of the methods adopted, or to the way these are used, is not a question that can be answered here. His arguments are based on formal language but are often illogical and imprecisely formulated. He purports to follow Popper but his theories are often unfalsifiable in principle or protected by phrases such as "Other things being equal". Large sections of the argument about relationships are indistinguishable from induction by enumeration and from the search for confirmation, as is shown by the repeated use of statements such as, "If we want to find support for the classification in Figure 4.12, we must look for characters in common between Caudata and Salientia".

The section which purports to confront his theory of vertebrate interrelationships with the facts of the fossil record, seems to rely heavily on the interpretation of the facts in the light of the theory they are said to support. Yet even this will not dispose of the Placodermi, which are therefore dismissed with an astonishingly vacuous quotation from Romer, which is said to seem "to be quite a close approximation to the truth". Unfortunately, the form of the argument is also imprecisely handled and this hinders comprehension. Some axioms are either self-contradictory or ambiguously stated, others are stated and then immediately refuted, and yet others introduced with an exception that is not defined until much later. At least one theory is a paraphrase of the definition from which it is said partly to be derived, and another is incompatible with the theory from which it is said to be deduced.

These are harsh criticisms of an honest attempt to deal in an explicit way with a number of difficult problems. I share Løvtrup's views on the importance of criticism and on the seriousness of the failure of many comparative biologists to make their assumptions, arguments and conclusions clear. Therefore, I recommend this book, particularly to those involved in tracing the history of life. For, despite its failures, it is full of challenging ideas, and I unreservedly commend the attitudes and hopes that motivated its writing.

R. S. Miles

R. S. Miles is Head of the Department of Public Services at the British Museum (Natural History).

Ellipsometry and polarised light

Ellipsometry and Polarized Light. By R. M. A. Azzam and N. M. Bashara. Pp. xvii+529. (North-Holland: New York, Amsterdam and Oxford, 1977.) Dfl.180; \$37.50.

ELLIPSOmetry is a technique for examining the optical properties of surfaces and thin films with polarised electromagnetic radiation. The technique is intrinsically very sensitive and deceptively simple in principle. Thus, it can readily be explained at a superficial level to students with only a minimal grasp of optics. Scientists who apply ellipsometry to investigate a wide range of phenomena, however, are aware of the need to take account of many possible sources of systematic error and of the consequent notorious difficulties in interpreting ellipsometric observations. The publication of a book of five hundred pages on ellipsometry will therefore not surprise them. Indeed, English-speaking scientists will welcome the first book of its kind written in their language by authors with international reputations for contributions to the development and use of ellipsometry.

The first two chapters are devoted to descriptions of well established and predominantly matrix methods of representing polarised radiation and the operation of optical elements with polarising properties. In chapter three the authors give an analysis of the main ellipsometric arrangements and a well ordered discussion of associated systematic errors; essentially the core

material of the book. The procedures for relating observable optical functions to the macroscopic optical constants of the materials of thin films and surfaces are presented in chapter four. Much of its contents can be found in other texts on thin film optics, but the account has been updated by references to recent work on anisotropic crystalline materials, as well as by a discussion of numerical inversion procedures. An account of instrumentation is given in chapter five with emphasis on imperfections and automation. In the final chapter, the authors provide a necessarily brief survey of the very wide range of physical surface investigation which has prompted the application of ellipsometry. Examples are taken from optics, physical chemistry, electrochemistry, particle radiation physics and biology.

The book is well written, with clear diagrams and few typographical errors. A pleasing feature of style are the separate introductions to each chapter, which provide the reader with a general preview of what is to follow. On the other hand, the balance of the material, which presumably reflects the authors' particular interests, will not be to the taste of some readers. For instance, the mathematical representations of polarised radiation are treated exhaustively at the expense of explanations of the underlying physical phenomena. A prominent example of inadequate emphasis concerns the ultimate sensitivity of ellipsometers; an important matter dismissed in a single paragraph.

P. H. Lissberger

P. H. Lissberger is Professor of Physics at the Queen's University, Belfast, Northern Ireland.

Ethological dictionary

Ethological Dictionary. By Armin Heymer. Pp. 237. (Paul Parey: Berlin and Hamburg, 1977.) DM28.

A dictionary in three languages (German, English, French) such as this one is useful to both students and research workers if it contains a wide enough selection of terms. I carried out a quick test by opening a recent textbook of animal behaviour at two randomly chosen pages. The Dictionary scored only a 40% success rate on the first ten technical terms encountered. Territoriality, echolocation, circadian rhythm and orientation were included and adequately defined, but habituation, phonoresponse, species recognition, arousal, sexual selection and selective attention were not listed.

Admittedly this is a small sample, but several of the missing terms are very common in ethological literature.

The dictionary does, however, score well in including some obscure terms with which I was not familiar. "Night dancers" are "bees that dance at 3.22 a.m. with only 12.5° error in the waggle dance", and equally intriguing "shag time" is apparently slang for "breeding season in hares and rabbits".

In general, the technical terms included in the dictionary and the way in which they are defined very much reflect the Lorenzian school of ethology, so that the dictionary might prove useful to those wishing to delve into the earlier German literature. Other users may find the range of terms covered too limited for the book to be worth buying as an all round aid to translation.

John Krebs

John Krebs is Lecturer in Zoology at the Edward Grey Institute of Field Ornithology, Oxford, UK.

Ecological stock-taking

A Nature Conservation Review: The Selection of Biological Sites of National Importance to Nature Conservation in Britain. Edited by D. A. Ratcliffe. Vol. 1. Pp. xvi+401. £35. Vol. 2. Pp. viii+320. £25. (Cambridge University: London, Cambridge and New York, 1977.)

THE many demands made on land in Britain are causing a widespread and significant reduction in wildlife. The purpose of *A Nature Conservation Review* has been to take stock of the nation's species and habitats and to identify those sites of national importance, so that new measures to safeguard them shall follow. The work was begun by the Nature Conservancy in 1965 and involved appraisal of several thousand sites. The process and its results are now published in two volumes.

Volume 1, which is complete in itself, explains the *Review's* purpose and methods, lists those sites which qualify as being of national importance and assesses how far their protection would ensure the survival of our total variety

of flora and fauna. It also provides a concise and detailed examination of British habitat types and their wildlife; this makes fascinating reading and will be invaluable for general reference.

Careful consideration is given to possible shortcomings in the *Review's* scope and methodology. This is of more than intrinsic value. There is a tendency not only in industry but amongst a few planning authorities and statutory agencies (who should know better) to doubt the Nature Conservancy Council's objectivity. *A Nature Conservation Review* will reassure them.

Volume 2 comprises descriptions of the 735 key sites, outlining the features which justify their selection from a total of some 3,500 scheduled over the years as Sites of Special Scientific Interest. Although many of the chosen sites contain rare species, good examples of typical habitats or wildlife communities are also included if the continued survival of the type is uncertain.

Obviously, there is room for discussion on whether the right sites were selected in every case but, accepting that new key sites may yet remain to be found and that others will change in merit, there can be little doubt that

the choice is largely correct. A pity then that, having taken ten years to publish the results of the survey, there has been some failure to amend it in the light of the greatly increased amount of information available in the mid-1970s. This is particularly evident where references occur to some rare birds whose recent status has changed notably.

It is particularly important that *A Nature Conservation Review* is read widely outside the nature conservation movement. Criticisms of details apart, it is a remarkable work which can do the NCC's standing nothing but good. It is also a means to an end: protection of the key sites must be achieved somehow and, as the NCC recognises, there is also a need to pursue more general measures for wildlife conservation throughout the countryside. At least volume one should be seen by all concerned with or by the use of land. It therefore passes comprehension that it has been priced so highly that it may fail to reach much of the readership at which it is aimed.

J. H. Andrews

J. H. Andrews is Head of the Conservation Planning Department at the Royal Society for the Protection of Birds.

Mammals of Pakistan

The Mammals of Pakistan. By T. J. Roberts. Pp. 361. (Ernest Benn: London and Tonbridge, 1977.) £35.

PAKISTAN lies astride the interface between the Palaearctic and Oriental faunal regions and, in addition, its fauna includes a small element from the Ethiopian region. The country is a mosaic of diverse habitats from the high montane snowlands of the Himalaya to the hot deserts east of the Indus. These factors are reflected in the richness of the mammal fauna, which includes over 150 species of recent mammals. Recent advances in agriculture and the increase in population have led to a rapid decline in numbers of the larger species and to an increased awareness of the role of smaller animals as pests and public health problems. An assessment of the current distribution and status of the mammals of Pakistan is therefore timely.

Tom Roberts has produced a comprehensive account of the mammals of the area which will be the standard work for years to come. The volume begins with two chapters on the zoogeography and habitats, and a chapter

on the adaptation of mammals to desert survival. The remainder of the book is devoted to a systematically arranged account of the species with information on description, distribution, status and biology. Keys are provided for the identification of each taxon. Maps, plates and drawings are used extensively to supplement the text. Useful appendices on field methods, a bibliography and a gazetteer are provided, and the book is fully indexed.

There are some weaknesses in the taxonomy which reflect the current lack of knowledge of Pakistan mammals and which cannot be attributed to any failure on the part of the author. The strength of the book lies in the author's painstaking compilation of data on distribution and status and his first-hand knowledge of the biology of the mammals based on his own field experience. The detailed treatment of each species surpasses the level expected in such a comprehensive volume.

The book should be of great value to the increasing number of mammalogists involved in agricultural, medical and conservation research in the area, as well as to the naturalist interested in the fauna of Pakistan and neighbouring regions.

I. R. Bishop

I. R. Bishop works in the Department of Zoology at the British Museum (Natural History).

**tins
will
change
your
reading
habits**
from July, 1978

ELSEVIER/NORTH HOLLAND
BIOMEDICAL PRESS

Circle No. 23 on Reader Enquiry Card.

Acridologists' vademecum

Grasshoppers and Locusts. Vol. 2. By Boris Uvarov. Pp. 613. (Centre for Overseas Pest Research: London, 1977.) £16.

ON retirement, Sir Boris Uvarov undertook the preparation of "a synthesis of present-day knowledge of the theory and practice of acridology", and this publication sees the eventual fulfilment of that task after his death in 1970. The first volume covering anatomy, physiology, development, phase polymorphism and an introduction to taxonomy came out in 1966 (Cambridge University Press). This second, posthumous volume covers behaviour (10 chapters) and ecology and biogeography (3).

The longest single chapter discusses the dynamics of grasshopper and locust outbreaks and plagues. Only in this chapter and an earlier section on swarm migrations is the information arranged by species; otherwise, it is organised by topics and truly comprehensive in scope. The book will thus be useful to many people who do not see themselves as 'acridologists' but wish to draw on the large store of information available from the research on these insects. They will not find all they want on any one topic within these 600 pages, 270 text-figures, 95 tables and 24 double-column pages of index, but they will get the gist of it along with a guide to the rest supported by 1,700 references.

Uvarov's colleagues Z. Waloff, R. F. Chapman and N. D. Jago, who edited the text after his death, have added supplementary material to his drafts but have not attempted to write the planned chapters he had not yet drafted, on economic aspects and the principles of control. Instead, they have rounded the book off with what amounts to a testament, a late review paper of Uvarov's on "Current and future problems of acridology", which sets forth his matured views on population dynamics, control and research.

The book may be fairly described as a monument to the author in more ways than one. The huge edifice of modern acridology was built on his inspiration and drive. The book is also very much the man, in its detailed attention to the facts, and to gaps in them, in its exceptional biological span, its scepticism, criticism and proposals for research, and in its sense of continuity between science and practice that Uvarov always displayed, not least in the unconventional naming of his research organisation, the "Anti-Locust Research Centre". As a more important illustration of that same point, the book incidentally goes some

way toward rehabilitating Uvarov's original discovery of phase transformation in locusts as the basis for a strategy of concentrating control operations on 'outbreak areas' where the swarming phase is produced from non-swarming precursors. Both ideas came under a cloud of criticism in the 1950s and 1960s, not least because it turned out that the most notorious species, the Desert Locust, was highly mobile in both phases and did not have just a few, permanent, outbreak areas like the others. By the time this volume was written, however, enough new information had accumulated to show that even in this species phase change or "gregarisation" is crucial in plague development and occurs in geographically identifiable areas, which are indeed larger and less fixed than the outbreak areas of other species but still far smaller than the

vast region subsequently over-run by the swarms.

Uvarov surely went too far in his reiterated hope that the outbreak areas would one day be deliberately altered ecologically for the sole purpose of preventing locusts from swarming, once and for all. Nevertheless, for the more pressing purposes of human settlement, the necessary ecological changes have already occurred in some regions such as North America, and they will doubtless occur in later-developing countries in due course. If the expected result is an endemic grasshopper problem replacing the locust invasions, we are fore-armed with this book.

J. S. Kennedy

J. S. Kennedy is External staff member, Agricultural Research Council, and Professor of Animal Behaviour at the Imperial College of Science and Technology, University of London, UK.

Calculating electronic structure

Semiempirical Methods of Electronic Structure Calculation. Part A (vol. 7) Techniques. Pp. 274. Part B (vol. 8): Applications. Pp. 274. Edited by G. A. Segal. (Plenum: New York and London, 1977.) \$47.40 each part.

WITH so many books available on molecular orbital theory it is hard to imagine many gaps in the literature. Nonetheless, with calculations being run increasingly by non-professional theoreticians there has been a need for a consumer guide to the various alternative semiempirical methods. To some extent the two volumes edited by Segal provide this service.

Most of the variants of molecular orbital techniques are the subjects of monographs but here in the first of the companion volumes several methods are summarised, each in about thirty pages. The individual contributors are distinguished advocates of the methods they describe; in particular the chapters on the PCIO and X α methods will be welcomed as providing more convenient summaries than those formerly available. One would wish, however, that for the price the coverage had been more comprehensive. Although neglect of differential overlap and the methods based on this approximation are the subject of one chapter, it would have been helpful to have a full chapter on the MINDO method even if the editor (as is clear from his preface) does not approve of this variant.

The tone of part A is serious and the theoretical basis for semi-empirical theories is discussed in the longest

chapter by Karl Freed. Many users of semi-empirical methods, on the other hand, only seek justification in the quality of predictions made by their calculations. Some of these applications are the subjects of the chapters in Part B.

Again, some well known names contribute. The topics to which the calculations are applied include thermochemistry; excited states of organic molecules; photochemistry; inorganic complexes; spin resonance parameters; solid-state problems; and electron scattering. Any non-specialist with a problem in one of these areas would be very much helped in choosing the most sensible approximation to select and given an honest indication of the quality of results he might expect.

In one respect the two volumes do not quite provide the complete consumer's guide. Many chemists are not controlled by the technique they use but by the molecules they are studying. They are interested in several properties of the same group of molecules, perhaps the vibration frequencies, the pK_a, the conformation and the nmr chemical shifts. A question one is often posed is, "Which method will answer these questions for me?" and at the same time "Just how much computer time is likely to be required?". A complete guide for the non-specialist would contain a set of 'benchmark' problems on model systems with the results from the various approximations compared and timed. Despite this omission the volumes will not rest unused on library shelves.

Graham Richards

Graham Richards is Lecturer in the Physical Chemistry Laboratory and a Fellow of Brasenose College, Oxford, UK.

announcements

On the Move

Professor F. Buchthal, Institute of Neurophysiology, Copenhagen, Denmark, to University Hospital (Rigshospitalet), Research Building, Copenhagen, from 1 September 1977.

Professor H. G. Schweiger, Max-Planck Institut für Zellbiologie, Wilhelmshaven, Germany, to Max-Planck Institut für Zellbiologie, Ladenburg, from 1 August 1977.

Dr J. Lawrence Fox, Associate Professor of Zoology, University of Texas at Austin, to Max-Planck Institut für Biochemie, Martinsried, Germany for one year sabbatical from 1 September 1977.

Dr B. P. Dash, Department of Geology, Imperial College of Science and Technology, University of London, to Chair of Applied Geophysics, University of Ife, Ife-Ife, Nigeria for two years from 1 September 1977.

Details of changes of department, sabbaticals, where leave will be taken and so on should be sent to *On the Move*; there is no charge for this service.

Appointments

Professor A. Lazenby, to Director of the Grassland Research Institute from 1 August 1977.

Dr R. J. Slater, to Director of Medical Programs for The National Multiple Sclerosis Society from 1 July this year.

Awards

Drs J. K. Burdett and **M. Poliakoff** are joint recipients of the Meldola Medal, awarded by the Royal Institute of Chemistry and Society of Maccabaeans for their work on laser chemistry and chemical effects of light.

The Actonian Prize of the Royal Institution has been awarded to **Professor R. L. Wain**, Professor of Agricultural Chemistry at Wye College, University of London and Honorary Director of the Agricultural Research Council Unit on Plant Growth substances and Systemic Fungicides, for his work on the chemical control of plant growth and the chemical basis of disease resistance in plants.

Dr F. Greaves, of the Imperial Cancer Research Fund has been awarded the Paul-Martini Prize 1977 (20,000 DM) for his work on a new diagnostic method for the specific proof of acute lymphoblastic leukaemia cells.

Meetings

14–16 September, **3rd National Quantum Electronics Conference**, Southampton (G. C. Thomas, Department of Electronics, University of Southampton, Highfield, Southampton, UK).

16–17 September, **Joint Radiological Meeting**, Guildford (The British Institute of Radiology, 32 Welbeck Street, London W1, UK).

19–23 September, **Application of Microprocessors**, Newcastle (The Institution of Electrical Engineers, Savoy Place, London WC2, UK).

Person to Person

A cooperative programme 'Species Pollution at World Scale' is trying to assess the real situation of the problem of introduced species. Botanists with experience in floristics are kindly requested to send the following information. (1) Place and area surveyed, (2) number of native species, (3) number of introduced species, including ephemerals and (4) a complete bibliographical reference for quotation, to E. H. Rapoport, Fundación Bariloche, c.c. 138, Bariloche 8400, Argentina. Collaborators will receive the printed map.

Exchange 4-bedroom furnished house in Montreal for similar in London area July 1978–August 1979. Car also considered. Contact D. Denhardt, 4338 West Hill Avenue, Montreal, Quebec H4B 2S9, Canada.

There will be no charge for this service. Send items (not more than 60 words) to Marcus Dobbs at the London office. The section will include exchanges of accommodation, personal announcements and scientific queries. We reserve the right to decline material submitted. No commercial transactions.

21 September, **Modern Trends in Fluorescence Techniques**, Guildford (D. Irish, Hon. Secretary, UV Group, c/o Pye Unicam Ltd, York Street, Cambridge, UK).

21–23 September, **The Safe Use of Materials**, Nottingham (R. H. Biddulph, Borax Consolidated Ltd, Cox Lane, Chessington, UK).

22 September, **Heterotechnologies for the Mail Service**, London (The Institution of Mechanical Engineers, 1 Birdcage Walk, London, UK).

26 September, **Surface Expression of Orebodies**, Swansea (The Institution of Mining and Metallurgy, 44 Portland Place, London, UK).

25–27 September, **Conference on Engineering for Health in Hot Countries**, Loughborough (R. Steele, University of Technology, Loughborough, UK).

25 September–11 October, **2nd International Kimberlite Conference**, Santa Fe, New Mexico (Kimberlite Conference, Sylvia-K Inc., 5671 Blue Sage Drive, Littleton, Colorado 80123).

26–28 September, **International Conference on Distributed Computer Control Systems**, Birmingham, UK (The Institution of Electrical Engineers, Savoy Place, London, UK).

26–28 September, **1st German Solar Energy Forum**, Hamburg (Congress Centrum Hamburg, Am Dammtor, PO Box 302360, D-2000 Hamburg 36, Germany).

26–29 September, **The 1st BOC Priestly Conference on Heterogeneous Oxidation**, Leeds (J. T. Gleave, Special Courses Division, Department of Adult Education and Extramural Studies, University of Leeds, Leeds, UK).

27–29 September, **International Conference on Power Electronics—Power Semiconductors and their Applications**, London (The Institution of Electrical Engineers, Savoy Place, London WC2, UK).

28 September, **Applications of Broad-Band Tuneable Lasers**, London (The Institute of Physics, 47 Belgrave Square, London SW1, UK).

28–30 September, **Symposium on Platelets: A Multidisciplinary Approach**, Florence, Fondazione Internazionale Menarini, Piazza del Carmine, 4-20121 Milan, Italy).

Reports and Publications Other countries—June

Annals of the South African Museum. Vol. 72, Part 12: The Validity of *Malacoraja* Stehmann, 1970; (Chondrichthyes, Batoidae, Rajidae) and Its Phylogenetic Significance. By P. Alexander Hulley and Matthias Stehmann. Pp. 227–237. R. 2. Vol. 72, Part 13: New Records of Marine Crustacea Isopoda from South Africa. By Brian Kensley. Pp. 239–265. R. 2.90. (Cape Town: South African Museum, 1977.) [216]
Office des Recherches sur les Pêcheries du Canada. Rapport Annuel 1976. Pp. 16. (Ottawa: Ministère des Pêches et de l'Environnement, Service des Pêches et de la Mer, 1976.) [226]
SRI. People at Work: Annual Report 1976. Pp. 32. (Menlo Park, California: SRI, 1977.) [226]
United States Department of the Interior: Geological Survey. Bulletin 1397-B: Mineral Resources of the Laramie Peak Study Area, Albany and Converse Counties, Wyoming. By Kenneth Segerstrom and

Robert C. Weisner. Pp. vi+35+plate 1. Water-Supply Pattern 2044: Modeling Chloride Movement in the Alluvial Aquifer at the Rocky Mountain Arsenal, Colorado. By Leonard F. Konikow. Pp. v+43. (Washington, DC: US Government Printing Office, 1977.) [276]

Proceedings of the Nutrition Society of Australia. Vol. 1: First Annual Conference, Melbourne, Victoria, August, 1976. Pp. 41. (Blacktown, NSW: Nutrition Society of Australia, Dr. J. L. Black (Hon. Secretary), c/o CSIRO, P.O. Box 239, 1977.) [273]

Space—Part of Europe's Environment Pp. 40. (Paris: European Space Agency, 8/10 rue Mario Nikis, 1977.) [276]

Deutsche Forschungsgemeinschaft, Bonn-Bad Godesberg. Tätigkeitsbericht 1976. Pp. 360. (Jahresbericht Band 1). Programme und Project 1976. Pp. 832. (Jahresbericht Band 2.) (Bonn-Bad Godesberg: Deutsche Forschungsgemeinschaft, 1977.) [286]

World Health Organization. Technical Report Series No. 606: The Role of Immune Complexes in Disease: Report of a WHO Scientific Group. Pp. 58. (Geneva: WHO, London: HMSO, 1977.) Sw. fr. 8; \$3.20. [286]

Harvard University: School of Public Health, Dean's Report, 1976. Pp. 56. (Boston, Mass.: Harvard University, School of Public Health, 1977.) [286]

Smithsonian Contributions to Zoology. No. 221: Polychaetes from Intertidal Areas in Panama, with a Review of Previous Shallow-Water Records. By Kristian Fauchald. Pp. iii+81. (Washington, DC: Smithsonian Institution Press, 1977. For sale by US Government Printing Office.) [286]

Fish Kills at Lake Lanier Hatchery: Investigations and Report of Findings. By W. C. Noell and G. B. Oglesby. Pp. iii+123. (Atlanta, Georgia: Department of Natural Resources. Environmental Protection Division, 270 Washington Street, SW, 1977.) [286]

Smithsonian Contributions to Zoology. No. 238: A Systematic Monograph of the Tongue Soles of the Genus *Cynoglossus* Hamilton-Buchanan (Pisces: Cynoglossidae). By A. G. K. Menon. Pp. iv+129 (21 plates). (Washington, DC: Smithsonian Institution Press, 1977. For sale by US Government Printing Office.) [296]

European Space Agency. ESA Annual Report 1976. Pp. 201. Report presented by the European Space Agency to the 20th COSPAR Meeting, Tel Aviv, Israel, June 1977. Pp. 268. (Paris: ESA, 1977.) [306]

Reports and Publications

UK & Ireland—July

Fulmer Annual Review, 1976. Pp. 8. Stoke Poges, Bucks: Fulmer Research Institute, Ltd., 1977. [17]

Smith Kline and French Foundation. Fourteenth Annual Report, 1976. Pp. 8. (Welwyn Garden City, Herts.: Smith Kline and French Foundation, 1977.) [47]

University of Oxford. Annual Report of the Curators of the Bodleian Library for 1975-76. (Supp. No. 4 to the *University Gazette*.) Pp. 48. (Oxford: The University, 1977.) £1.75. [47]

Microbiological Research Establishment. Abstracts of Work Published in 1976. Pp. 22. (Porton Down, Salisbury, Wiltshire: Microbiological Research Establishment, 1977.) [57]

National Radiological Protection Board. NRPB-R52: Human Exposure to Radiation Following the Release of Radioactivity from a Reactor Accident—a Quantitative Assessment of the Biological Consequences. By H. Smith and J. W. Stather. Pp. ii+39. NRPB-R60: Measurement of Activity of Surfaces Contaminated by Beta-emitting Nuclides and ^{59}Fe . By W. J. Iles, P. H. Burgess and D. F. White. Pp. 13. (Harwell, Didcot, Oxon: National Radiological Protection Board, 1977.) [57]

Chemical Industries Association. Labelling Chemicals: a Guide to the Regulations for the Labelling and Marking of Containers and Vehicles. Pp. 133. (London: Chemical Industries Association, Ltd., 93 Albert Embankment, SE1, 1977.) [57]

Wye College: Department of Hop Research—Annual Report from 1 April 1976 to 31 December 1976. (University of London.) Pp. 68. (Nr. Ashford, Kent: Wye College, 1977.) 80p. [67]

Tropical Storage Abstracts, No. 1, 1977. Pp. 14. (Slough: Tropical Stored Products Centre (Tropical Products Institute), London Road, 1977.) [67]

Third Special Report from the Select Committee on Science and Technology, Session 1976-77. Pp. ii+2. (London: HMSO, 1977.) 15p net. [67]

Philosophical Transactions of the Royal Society of London. A: Mathematical and Physical Sciences. Vol. 286, No. 1334: Turbulence and Mixing in a Scottish Loch. By S. A. Thorpe. Pp. 125-181+plate 1. (London: The Royal Society, 1977.) UK £3.60; Overseas £3.80. [67]

Office of Population Censuses and Surveys. Population Trends 8, Summer 1977. Pp. 62. (Government Statistical Service.) (London: HMSO, 1977.) £2 net. [77]

An Appraisal of Various Approaches to the Fractionation of Tobacco Smoke Condensate. By J. K. Whitehead. (Research Paper 13.) Pp. 56. (London: Tobacco Research Council, Glen House, Stag Place, SW1, 1977.) [77]

Grassland Research Institute. Annual Report for 1976. Pp. xvi+172. (Hurley, Maidenhead, Berkshire: The Grassland Research Institute, 1977.) £2.50. [77]

Building Research Establishment. Research Programme 1977-78. Pp. 48. (Garston, Watford: Building Research Establishment, Department of the Environment, 1977.) [77]

Cumbria: Freshwater Biological Association, 1977.) £2. [87]

Scottish Plant Breeding Station. Fifty-Sixth Annual Report, April 1976 to March 1977, and the Report of the Scottish Society for Research in Plant Breeding. Pp. 132. (Pentlandsfield, Roslin, Midlothian: Scottish Plant Breeding Station, 1977.) [87]

Computer-Based Packages for Teaching Earth Science. Report No. 5: Programs in BASIC for Simple Correlation and Regression (With Worked Examples). By Roger Till (with programs by Sue Watts). Pp. 62. (Reading University Geological Reports, No. 11.) (Reading: Geology Department, The University, 1977.) 75p. [87]

Natural Environment Research Council: Institute of Terrestrial Ecology. Culture Centre of Algae and Protozoa—List of Strains 1976. Pp. ii+120. £1 net. A Coded Checklist of Animals Occurring in Fresh Water in the British Isles. By Dr. P. S. Maitland. Pp. 76. £1.50 net. Ecology of Red Deer: a Research Review Relevant to Their Management in Scotland. By Brian Mitchell, Brian W. Staines and David Welch. Pp. vii+74. £2 net. Atlas of the Non-Marine Mollusca of the British Isles. Edited by M. P. Kerney. Pp. v+199. £3. (Cambridge: The Institute of Terrestrial Ecology, 68 Hills Road, 1976 and 1977.) [87]

Safety Representatives and Safety Committees. (Health and Safety at Work.) Pp. 46. (London: Health and Safety Commission, 1977. Available from HMSO.) 35p. [117]

Department of Health and Social Security. Welsh Office. Residential Homes for the Elderly: Arrangements for Health Care. (A Memorandum of Guidance.) Pp. 20. (London: DHSS, 1977.) [117]

Ministry of Agriculture, Fisheries and Food: Directorate of Fisheries Research. Fisheries Radiobiological Laboratory. Technical Report FRL 12: Radioactivity in Surface and Coastal Water of the British Isles, 1975. By N. T. Mitchell. Pp. 32. (Lowestoft: Fisheries Radiobiological Laboratory, 1977.) [147]

Queen Mary College, University of London. Annual Report, Session 1975-76. Pp. 63. (London: Queen Mary College, Mile End Road, E1, 1977.) [147]

Royal Greenwich Observatory. Bulletin, No. 182: The Galaxy and the Local Group. (Tercentenary Symposium held at Herstmonceux Castle, 1975, July 22-25.) Edited by R. J. Dickens and Joan E. Perry. Pp. xi+293. £7.50 net. Report 1975/76. Pp. 64. £1 net. (Herstmonceux: Royal Greenwich Observatory, 1976 and 1977.) [147]

Council for National Academic Awards. Annual Report for 1976. Pp. 71. (London: Council for National Academic Awards, 344/354 Gray's Inn Road, WC1, 1977.) [147]

The Radiochemical Centre, Amersham. Radiochemicals 1977/8. UK edition. Pp. 143. (Amersham: The Radiochemicals Centre, 1977.) [147]

Laboratory of the Government Chemist. Report of the Government Chemist 1976. Pp. iv+166. (London: HMSO, 1977.) £3 net. [157]

Science Research Council. The Work of the Rutherford Laboratory, 1976. Edited by Gordon Fraser. Compiled by the Laboratory Information Panel. Pp. xii+127. (Chilton, Didcot, Oxon: Science Research Council, Rutherford Laboratory, 1977.) [187]

Ministry of Agriculture, Fisheries and Food: Directorate of Fisheries Research. Fisheries Radiobiological Laboratory. Technical Report FRL 12: Radioactivity in Surface and Coastal Water of the British Isles, 1975. By N. T. Mitchell. Pp. 32. (Lowestoft: Fisheries Radiobiological Laboratory, 1977.) [187]

Chinese Astronomy, Vol. 1, No. 1, June 1977. (A selected translation of *Acta Astronomica Sinica*.) Pp. 1-196. Published Bi-Annually. Annual subscription: \$105. (Oxford and New York: Pergamon Press, 1977.) [187]

Nuclear Track Detection, Vol. 1, No. 1, 1977. Pp. 1-80. Editor-in-Chief: S. A. Durrano. Published Quarterly. Annual subscription: \$55. (Oxford and New York: Pergamon Press, 1977.) [187]

Philosophical Transactions of the Royal Society of London. B: Biological Sciences. Vol. 279, No. 969: A Physiological, Biochemical and Histological Study of Goose Tracheal Mucin and Its Secretion. By R. J. Phipps, P. S. Richardson, A. Corfield, J. T. Gallagher, P. K. Jeffrey, P. W. Kent and M. Passatore. Pp. 513-543+plates 1-4. (London: The Royal Society, 1977.) UK £2.90; Overseas £3. [207]

The Royal Scottish Museum Triennial Report, 1974-76. Pp. 74+12 plates. (Edinburgh: The Royal Scottish Museum, 1977.) £4 net. [227]

Indexed Bibliography of Publications on Water and Waste Engineering for Developing Countries. Edited by John Pickford. Pp. 52. (Loughborough: WEDC Group, Department of Civil Engineering, University of Technology, 1977.) £7. [257]

National Coal Board. Report and Accounts, 1976/77. Pp. 76. Statistical Tables, 1976/77. Pp. 12. (London: National Coal Board, 1977.) [257]

Philosophical Transactions of the Royal Society of London. A: Mathematical and Physical Sciences. Vol. 286, No. 1335: Steep Gravity Waves in Water of Arbitrary Uniform Depth. By E. D. Cokelet. Pp. 183-230. (London: The Royal Society, 1977.) UK £3.20; Overseas £3.35. [257]

Department of Industry, Industry, Education and Management—a Discussion Paper. Pp. ii+78. (London: Department of Industry, 1 Victoria Street, SW1, 1977.) [257]

British Nuclear Fuels Limited. Sixth Annual Report and Accounts, 1976/1977. Pp. 31. (Risley, Warrington: British Nuclear Fuels Limited, 1977.) [267]

Making Success Happen: Enterprise and Change in British Chemicals. Pp. 8. (London: Chemical Industries Association Limited, 93 Albert Embankment, SE1, 1977.) [267]

Chemical Crosswords: a Learning Aid for Chemistry

Students, 20 crosswords. By A. G. Hudson and S. Hind. (Tettenhall, Wolverhampton: Sigma Technical Press, 23 Dippons Mill Close, 1977.) £1. [267]

British Gas Corporation. Annual Report and Accounts, 1976/1977. Pp. 72. (London: British Gas Corporation, 59 Bryanston Street, W1, 1977.) [267]

The Association of the British Pharmaceutical Industry. Annual Report, 1976/1977. Pp. 44. (London: The Association of the British Pharmaceutical Industry, 162, Regent Street, W1, 1977.) [267]

Proceedings of the Royal Society of London. A: Mathematician and Physical Sciences. Vol. 355, No. 1683: A Discussion on New Particles and New Quantum Numbers. Organized by R. H. Dalitz, FRS, B. Richter, B. Wiik and W. T. Toner. Pp. 441-637+1 plate. (London: The Royal Society, 1977.) UK £4.85; Overseas £5. [277]

Social Science Research Council. Energy Topics in the Social Sciences: a Report to the Research Initiatives Board of the Social Science Research Council. Pp. 13+ix. (London: Social Science Research Council, 1 Temple Avenue, EC4, 1977.) [277]

The Macaulay Institute for Soil Research. Annual Report for 1975/1976. (No. 46.) Pp. 134. (Craigiebuckler, Aberdeen: The Macaulay Institute for Soil Research, 1977.) [277]

Memoirs of the Royal Astronomical Society. Vol. 84, Part 1: Accurate Position Measurements and Optical Identifications for Radio Sources Selected at 966 MHz. By A. M. Cohen, R. W. Porcas, L. W. A. Browne, E. J. Daintree and D. Walsh. Pp. 1-44. (Oxford and London: Blackwell Scientific Publications, 1977. Published for The Royal Astronomical Society.) [287]

Reports and Publications

Other countries—July

Prospective et Santé, No. 1. Revue Trimestrielle. Pp. 1-141. Rédacteur en Chef: Michael Salomon. (Paris: *Prospective et Santé*, 5, rue Clément Marot, 1977.) [17]

Smithsonian Contributions to Zoology. No. 237: The Pierid Butterflies of the Genera *Hypocholia* Ureta, *Phulia* Herrich-Schäffer, *Infraphulia* Field, *Pierphulia* Field, and *Piercolias* Staudinger. By William D. Field and José Herrera. Pp. iii+64. (Washington, DC: Smithsonian Institution Press, 1977. For sale by US Government Printing Office.) [17]

United States Department of the Interior: Geological Survey. Professional Paper 880: Geology of the Apollo 14 Landing Site in the Fra Mauro Highlands. By G. A. Swann, et al. Pp. vii+103+plates 1-7. (Washington, DC: US Government Printing Office, 1977.) [47]

National Research Council Canada. NRS Associate Committee on Scientific Criteria for Environmental Quality. The Effects of Alkali Halides in the Canadian Environment. Pp. 171. (Ottawa: National Research Council Canada, 1977.) \$2. [47]

Geological Survey of Canada. Bulletin 281: Taxonomy of Upper Jurassic—Lower Cretaceous Microplankton from the Richardson Mountains, District of Mackenzie, Canada. By W. W. Bideaux. Pp. 89 (16 plates). (Ottawa: Geological Survey of Canada, 1977.) \$5. [47]

World Armaments: The Nuclear Threat. Pp. 39. (Stockholm: Stockholm International Peace Research Institute, 1977.) [57]

Development and Evaluation of a Hexokinase/Glucose-6-Phosphate Dehydrogenase Procedure for Use as a National Glucose Reference Method. By James W. Neese, Patricia Duncan, David Bayse, Mary Robinson, Teresa Cooper and Charles Stewart. Pp. vi+147. (Atlanta: US Department of Health, Education and Welfare, Public Health Service, Center for Disease Control, Bureau of Laboratories, Clinical Chemistry Division, 1976.) [57]

European Organization for Nuclear Research—CERN. Annual Report, 1976. Pp. 177. (Geneva: CERN, 1977.) [57]

Bulletin of the American Museum of Natural History. Vol. 158, Article 5: Stratigraphy and Biostratigraphy of Late Cenozoic Deposits in Central Sioux County, Western Nebraska. By Morris F. Skinner, Shirley M. Skinner and Raymond J. Gooris. Pp. 263-370. \$7.60. Vol. 158, Article 1: A Revision of the Spider Genera *Herpyllus* and *Scotophaeus* (Araneae, Gnaphosidae) in North America. By Norman I. Platnick and Mohammad U. Shadab. Pp. 1-44. \$2.80. (New York: American Museum of Natural History, 1977.) [67]

Annals of the South African Museum. Vol. 73, Part 2: Relationships of the South African Fossil Frog *Ecoenopoides reuningi* (Anura, Pipidae). By Richard Estes. Pp. 49-80. R.3. Vol. 73, Part 3: Cretaceous Deposits Near Bogenfels, South West Africa. By Herbert Christian Klinger. Pp. 81-92. R.1.90. Vol. 73, Part 4: Deep-Sea Amphipods from West of Cape Point, South Africa. By Charles L. Griffiths. Pp. 93-104. R.1.90. Vol. 73, Part 5: The South African Museum's *Meiring Naude* Cruises. Part 3: Hydrozoa. By N. A. H. Millard. Pp. 105-131. R.2.80. Vol. 73, Part 6: The South African Museum's *Meiring Naude* Cruises. Part 4: Echinoderms. By Ailsa M. Clark. Pp. 133-147. R.2.20. (Cape Town: South African Museum, 1977.) [77]

United States Department of the Interior: Geological Survey. Professional Paper 1018: Characteristics of Thin-Skinned Style of Deformation in the Southern Appalachians, and Potential Hydrocarbon Traps. By Leonard D. Harris and Robert C. Millic. Pp. iv+40+9 plates. (Washington, DC: US Government Printing Office, 1977.) [77]

International Development Research Centre, Ottawa. Agriculture, Food and Nutrition Sciences Division: The First Five Years. Pp. 48. (Ottawa: International Development Research Centre, Box 8500, 1977.) [87]